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- 3 Mann, T. The Biochemistry of Semen and of the Male Reproductive Tract. Methuen, London, 1951.
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Acta Obstetricia et Gynecologica Scandinavica

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- 1 Cruickshank, J. M. Stuart Smith & A. Orchus associated with spermagglutinating antibodies. *Lancet* 1 708 1959
- 2 Fjalbrant, B. Immunoagglutination of sperm in cases of sterility. *Acta Obstet Gynecol Scand* 44 474 1965
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TUBO-OVARIAN ABSCESS IN PREGNANCY¹

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Abstract A case of tubo-ovarian abscess in a patient with pregnancy is reported. Nineteen reports of ovarian abscesses are collected from the literature and analyzed. The treatment is discussed.

Acute and chronic pelvic inflammatory disease during pregnancy is seldom encountered. Differential diagnosis of pelvic pain with signs of inflammation during pregnancy includes abortion, especially minimal abortion, appendicitis, bowel obstruction, pregnancy and twisted ovarian cyst. Because of its rarity, tubo-ovarian inflammatory disease is not often considered. This diagnosis is usually made after laparotomy.

Most reported cases of pelvic inflammatory disease have occurred in early pregnancy. There has been only one report of term pregnancy complicated by an ovarian abscess in the English literature.

The following is a case report of a patient with an asymptomatic tubo-ovarian abscess and a term pregnancy.

CASE REPORT

A 34-year-old black female, gravida 3, para 2-1-0-1 at 41 weeks gestation was admitted to Obstetric Service at Cook County Hospital on July 18, 1974, complaining of mild labor pains.

She had prenatal care at the Board of Health Clinic and had no complications. She gave a history of oophorectomy three months after her second delivery in 1969. At that time she was told that her tubes were bad and she would not be able to get pregnant again. The patient used no contraception since 1969 and said she had never been treated for gonorrhea or other pelvic inflammatory diseases.

At the time of hospital admission her blood pressure

was 160/100 mmHg, pulse 88/min, temperature 98.4°F. Fetal heart tones were 147/min. Uterus was term size. Cervix was closed and vertex was 2 cm above the ischial spines. There was moderate pitting edema of the lower extremities.

As the patient was not in labor and because of hypertension it was decided to induce the labor. During the course of induction a fetal distress developed and an emergency low transverse cesarean section was performed. A male infant, appar 7 weighing 7 lbs 14 oz was delivered. Her right tube and ovary were found to be normal. In the left adnexal area there was a 12×12 cm abscess adherent to the posterior surface of the uterus, lateral pelvic wall and sigmoid colon. The abscess wall was inadvertently ruptured during the process of dissection and approximately 30 cc of pus was evacuated.

The patient desired sterilization but since her condition precluded hysterectomy at this time a left salpingo-oophorectomy and right Pomeroy tubal ligation was performed.

Patient was given antibiotics and had an afebrile postoperative course. She was discharged with her baby on the 9th postoperative day, both in good condition.

Histopathologic examination of the surgical specimen confirmed the diagnosis of ovarian abscess and chronic salpingitis. Culture of the abscess grew *Peptococcus*.

DISCUSSION

The etiology of pelvic inflammatory disease in pregnancy has been suggested by most authors (2, 7, 9, 10, 12, 19) as follows:

- 1) Lymphatic spread from vagina or cervix
- 2) Hematogenous spread from distant organs
- 3) Flare-up of a pre-existing infection of the tube or ovary
- 4) Spread by continuity from an adjacent focus such as the appendix
- 5) Ascending infection about the time of conception

Acute pelvic inflammatory disease during pregnancy is extremely rare. Occasional reports in the literature are not well documented. However

¹ Presented at the Chicago Gynecological Society Meeting in Chicago, Illinois, November 1974.

Table I Summary of documented tuboovarian abscesses in pregnancy

TO=tuboovarian TOA=tuboovarian abscess OA=ovarian abscess TAH=total abdominal hysterectomy
bilateral salpingo-oophorectomy vs =versus R=right L=left RO=right oophorectomy SO=salpinge
CS=cesarean section NSVD=normal spontaneous vaginal delivery AB=abortion

Case	Length gestat	Symptoms and signs					Preoperative diagnosis
		Pain	Temperature	Peritonitis	Mass	Prior PID	
Aikens 1870 (7)	28 w					Yes	
Brindeau 1917 (3)	term	Yes	Yes		No		
Cron 1924 (5)	3½ mo	Yes	Low grade	No	Yes		TO mass
Mocquot 1939 (3)	2½ mo	Yes pelvic	Yes		Yes	No	
Palmer 1939 (3)	1½ mo	Yes	Yes		Yes		
Merajkar 1940 (17)	7 mo	Yes pelvic	Yes	Yes	Yes	No	Periappendicular abscess
McCall 1948 (15)	4 mo						
Cummin 1951 (6)	20 w	Yes pelvic	No	Yes	No	No	Torsion of ovarian cyst vs periappendicular abscess
Lowne et al 1951 (14)	7 mo	Yes pelvic	Yes	Yes			Abruptio placentae
Jzynski et al 1954 (4)	20 w	No	No	No	Yes	Yes	Ovarian cyst
1957 (11)	5 mo	Yes pelvic	No	No	Yes	Yes	Pelvic mass
Powell et al 1958 (18)	7 mo						
Evans 1959 (8)	25 w	Yes pelvic	Yes		No		Degenerating fibroid
Friedman 1959 (9)	6 w	Yes	Low grade	No	Yes	Yes	Ectopic pregnancy vs torsion of ovarian cyst
	28 w	Yes abdominal	No	Yes	No	No	Abruptio placentae vs torsion of ovarian cyst
Baydoun et al 1961 (?)	14 w	Yes abdominal	No	Yes	Yes	Yes	
Dudley et al 1970 (7)	17 w	Yes pelvic	Low grade	Yes	Yes	Yes	Ruptured TOA
Hunt et al 1974 (10)	25 w	Yes abdominal	No	No	No	Yes	Abruptio placentae
Jafari et al 1975	41 w	No	No	No	No	Yes	Fetal distress

Findings	Ruptured	Side	Opposite adnexa	Procedure	Fate of gestation	Maternal death	Microorganism
Normal tube	Yes	R	Normal	Autopsy		Yes	
		R		RO	NSVD prior to laparotomy	Yes	
Size of large	No	L		LSO	AB same day	No	Sterile
Purulent L. inguitis		L	Normal	LSO	Term NSVD	No	
Salpinx	No	L	Normal	LSO	AB after 36 hours	No	
Content of ova and Fallopian tissue		R		1) abd drainage 2) abd drainage excochleation	Induced delivery prior to surgery		Streptococcus
Size of uterine tube	Yes	R	Normal	RSO	AB Spontaneous AB after 12 hours	No	Anaerobic streptococcus
Involving in abruptio placentae	Yes	R	Clubbed L. tube normal ovary	CS RSO + cornual resection	Premature del by CS exp after 48hrs	No	E coli
8 cm	No	R	Normal	RSO	Term NSVD supervised	No	Pneumococcus
Normal tube	No	R	Normal	RO	Term NSVD survived	No	Alpha hemolytic streptococcus
				CS SO	Neonatal death	No	
Uterus didelphys pregnancy in cornu	Yes	R	Normal	Autopsy		Yes	Anaerob strep Gram negative bacilli
cm	No	R	Normal	RSO	AB 10 wks later	No	Streptococcus
1 cm subacute salpingitis	No	R	Normal	RSO	Term assisted breech delivery survived	No	Sterile
4-16 cm chronic abscess	Yes	R	Normal	RO drainage	AB after 9 days	No	Strep fecalis E coli
	Yes	R	Normal	TAH BSO	AB during surgery	No	Sterile
10-17 cm 3-4 cm	No	Bil		CS subtotal hysterectomy by BSO	Premature del by CS survived	No	Proteus mirabilis
cm adherent ended tube	No	L	Normal	CS LSO	Postmature del by CS survived	No	Peptococcus

there are 7 cases reported in the English literature where acute salpingitis was observed at laparotomy. The disease appeared usually during the 1st trimester; the most advanced reported was in a patient of 24 weeks gestation (1, 2, 3, 16, 19).

Tubo-ovarian and ovarian abscesses in pregnancy are also rare. We have found only 19 cases described in the literature; some are not described in great detail but all are documented by laparotomy. These reports are summarized in Table 1 (2, 11, 14, 15, 17, 18).

There were 6 ovarian abscesses, 3 ovarian abscesses with some tubal involvement and 10 tubo-ovarian abscesses.

In contrast to acute salpingitis the abscesses were rather evenly distributed throughout the pregnancy: 6 were diagnosed in the 1st trimester, 8 in the 2nd and 5 in the 3rd. Only 2 were found at term.

The symptoms of this condition are variable. The most constant symptom was pain. Two patients were asymptomatic. One patient was operated on because of a pelvic mass (4); the other abscess was an incidental finding at the time of cesarean section. Elevation of temperature was noted in about one half of the patients and it was usually of the low grade.

In only one instance was the diagnosis made operatively (7). It was a ruptured tubo-ovarian abscess in which the mass was palpable and ultrasound revealed pus.

Cultures of the pus most frequently grew streptococcus or anaerobic streptococcus. However, 3 out of 13 cultures were sterile.

There were 3 maternal deaths, 2 of which occurred prior to the antibiotic era.

In the management of chronic tubo-ovarian inflammatory disease with abscess formation in non-pregnant patients, most authors seem to agree that an aggressive approach by surgical removal of the uterus, tubes and ovaries reduces the need for a second operation and the risk of other complications (3).

The data on chronic tubo-ovarian disease in pregnancy are too limited to draw any conclusions about therapy. As shown in Table 1, there were 9 cases of tubo-ovarian abscess in pregnancy managed by conservative surgery, i.e. removal of the tube and/or ovary. Five of these patients aborted spontaneously and 4 pregnancies were carried to term. Therefore, conservative surgery in selected cases seems to be appropriate.

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THE INFLUENCE OF SMOKING ON THE HAEMOSTATIC MECHANISM IN PREGNANCY

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Abstract A prospective study through pregnancy is described comparing smokers with non smokers in an attempt to assess possible changes in the haemostatic mechanism. Various components of the coagulation and fibrinolytic enzyme systems were assayed serially up to 38 weeks of gestation. Plasma fibrinogen was lower in the smokers than in the non-smokers. Also plasminogen was slightly decreased and plasminogen activator activity and serum FDP increased in the former group. Although the results failed to demonstrate major disseminated intravascular coagulation in smokers the pattern of a possible low grade syndrome emerged.

It has now been clearly established that smoking in pregnancy results both in a reduced incidence of pre-eclampsia and low birth weight babies (6, 18, 23). The mechanisms involved are unknown but could be related to the nicotine content of the cigarettes: the smoking of one cigarette giving the equivalent of an intravenous dose of 1-2 µg per kg body weight (2). Also nicotinic acid has the property of inducing intense but transient fibrinolytic activity when injected into normal adults (19). In addition smoking is known to have widespread effects on the haemostatic mechanism in the direction of increased coagulation (11, 1).

Since abnormalities of the haemostatic mechanism have been mooted in the pathogenesis of both pre-eclampsia and fetal growth retardation (4) it was decided to compare prospectively through pregnancy smokers with non smokers by assaying various components of the coagulation and fibrinolytic enzyme systems.

SUBJECTS AND METHODS

Twenty four normal primigravidae were selected at 11 weeks of pregnancy. All had a uterine size compatible with the period of amenorrhoea. The age range was 18-35

years; heights were 152 cm or over; weight/height ratios were in the interquartile range (8) and there was no history of abortion, hypertension or renal disease. All progressed normally and delivered normal full term infants. Twelve of the subjects were non smokers and 12 habitually smoked 10 or more cigarettes daily. Their smoking habits were unchanged throughout the study.

Blood was collected aseptically at 12, 20, 25, 30, 34 and 38 completed weeks of gestation. Plasma samples were collected into sodium citrate 3.8% solution in plastic tubes; serum samples were collected into glass tubes and allowed to stand for one hour before separation. Plasminogen activator assays were carried out within 70 minutes of collection and plasma and serum were stored in aliquots at -20 °C for the other assays.

The following parameters were measured:

Fibrinogen—measured in plasma and expressed as mg/100 ml (Ratnoff & Menzie (16)).

Plasminogen—measured in plasma and expressed as casein units/ml (Remmert Cohen (17)).

Plasminogen Activator—measured in plasma according to the method of Nilsson & Olow (12) but converted on a log-log graph to units of activity, 500 minutes being assigned one unit (7).

Serum Fibrin Degradation Products (FDP)—expressed as µg/ml (Merskey et al (10)). The blood had been collected into plastic tubes containing glass beads and aprotinin (Trasylol) 0.1 ml and incubated at 37°C for at least 3 hours before separation in order to inhibit *in vitro* fibrinolysis.

Antithrombin III—measured in serum by radial immunodiffusion using Nygaard standard serum and expressed as "normal" (Mancini et al (9)).

α1 Antitrypsin and α2 Macroglobulin—measured as for Antithrombin III using Partigen Plates (Hoechst) and expressed as mg/100 ml.

RESULTS (TABLE I)

Using Student's *t* test the only statistical difference recorded below the 5% level of significance was fibrinogen at 20 weeks of gestation which was lower in the smoking group (*p* < 0.05).

Table I Components of the haemostatic mechanism in pregnancy

Smokers compared with non smokers Means \pm 1 S D are recorded at each completed week of gestation

Parameter		12 weeks	20 weeks	25 weeks	30 weeks	34 weeks	38 w
Fibrinogen mg/100 ml	Non-smokers	360 \pm 95	370 \pm 60	385 \pm 66	441 \pm 82	500 \pm 73	483 \pm 7
	Smokers	321 \pm 41	316 \pm 47	352 \pm 68	409 \pm 65	450 \pm 108	448 \pm 7
Plasminogen casein units/ml	Non smokers	4.5 \pm 0.5	4.8 \pm 0.3	4.8 \pm 0.4	4.9 \pm 0.4	4.8 \pm 0.6	4.9 \pm 0.4
	Smokers	4.2 \pm 0.6	4.4 \pm 0.6	4.5 \pm 0.7	4.5 \pm 0.7	4.6 \pm 0.5	4.7 \pm 0.4
Plasminogen activator units of activity	Non smokers	3.2 \pm 1.3	1.3 \pm 0.6	—	0.9 \pm 0.4	—	0.9 \pm 0.4
	Smokers	3.1 \pm 1.4	1.6 \pm 0.5	—	1.2 \pm 0.7	—	0.9 \pm 0.4
Serum F D P μ g/ml	Non-smokers	4.4 \pm 5.0	4.6 \pm 1.7	5.1 \pm 4.8	5.8 \pm 4.0	6.7 \pm 4.1	4.7 \pm 4.4
	Smokers	6.5 \pm 4.4	6.4 \pm 4.2	12.4 \pm 9.5	7.6 \pm 3.5	7.7 \pm 4.7	10.7 \pm 4.1
Antithrombin III % normal	Non smokers	105 \pm 35	128 \pm 48	97 \pm 26	110 \pm 28	107 \pm 37	96 \pm 3
	Smokers	123 \pm 26	108 \pm 52	106 \pm 25	117 \pm 61	100 \pm 23	109 \pm 23
α 1 antitrypsin mg/100 ml	Non smokers	351 \pm 116	452 \pm 99	398 \pm 115	418 \pm 55	416 \pm 91	474 \pm 7
	Smokers	377 \pm 148	384 \pm 124	431 \pm 121	387 \pm 140	400 \pm 103	438 \pm 10
α 2 macroglobulin mg/100 ml	Non-smokers	382 \pm 120	429 \pm 154	349 \pm 126	372 \pm 131	366 \pm 147	349 \pm 10
	Smokers	446 \pm 87	458 \pm 120	444 \pm 128	428 \pm 172	396 \pm 108	479 \pm 1

Although no valid statistical comparison could be made between smokers and non smokers at individual gestations with regard to changes in the haemostatic mechanism various apparent patterns of difference emerged (Fig. 1)

Mean fibrinogen and plasminogen levels were slightly lower throughout pregnancy and reached a lower peak in the smoking group

Fibrinolytic activity fell in the smokers to an equally low level by 38 weeks of pregnancy but at a slower rate

Serum F D P were slightly higher at each point in the smoking group

α 2 macroglobulin was consistently slightly higher in the smoking group

No pattern of difference emerged for the other anti plasmin α 1 antitrypsin or for antithrombin III

The patterns of difference were tested longitudinally over pregnancy for each individual parameter using Cochran's method of summation of binary probabilities (5) and the results were highly significant

Fibrinogen	$p < 0.0001$
Plasminogen	$p < 0.0001$
Plasminogen activator	$p < 0.01$
F D P	$p < 0.001$
α 2 Macroglobulin	$p < 0.0001$

Similarly a highly significant pattern emerged on vertical testing at each gestation of the following parameters fibrinogen plasminogen plasminogen activator and F D P

12 weeks	$p < 0.01$
20 weeks	$p < 0.0001$
25 weeks	$p < 0.005$
30 weeks	$p < 0.001$
34 weeks	$p < 0.005$
38 weeks	$p < 0.001$

DISCUSSION

The pattern of changes observed over pregnancy in both groups of subjects was characteristic of changes in the haemostatic mechanism in normal pregnancy with an altered dynamic equilibrium between coagulation and fibrinolysis in favour of systemic fibrin deposition (3, 4)

This study failed to demonstrate major disseminated intravascular coagulation in the smoking group but significant although transient phases of fibrin deposition associated with the smoking of individual cigarettes could have been overlooked

The lower fibrinogen in the smoking group may have been due to a slow intravascular coagulation and fibrinolysis or to reduced synthesis of fibrinogen

The significant pattern of decreased plasminogen

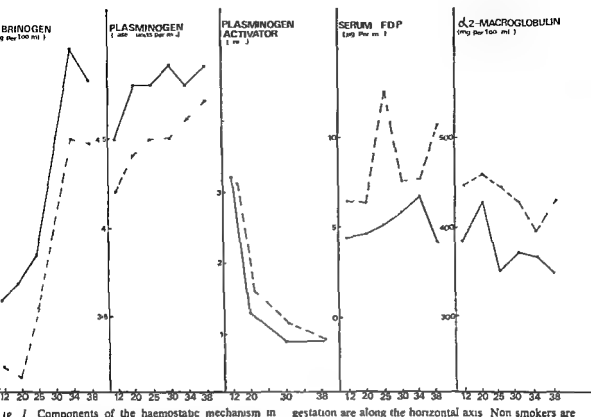


Fig. 1 Components of the haemostatic mechanism in pregnancy. Smokers compared with non smokers. Mean values for each parameter are depicted. Units of measurement are on the vertical axis and completed weeks of

gestation are along the horizontal axis. Non smokers are depicted by a continuous line and smokers by a broken line.

en with increased plasminogen activator activity and increased FDP is compatible with fibrinolysis. However a low grade of intravascular coagulation in association with secondary fibrinolysis could not be confirmed in the absence of such determinations as platelet counts and examination of fibrin monomers (14). Also using more sophisticated methods it would have been possible to ascertain whether the FDP originated from brin or fibrinogen (15).

The higher antiplasmin level in the smoking group could have prevented further fibrinolytic activity thus representing a possible element of physiological compensation.

A low grade intravascular coagulation in pregnancy influenced by smoking is difficult to reconcile with the coagulation theory for the pathogenesis of pre eclampsia in view of reports that smoking protects against this syndrome. However such a coagulopathy would be compatible with the platelet insufficiency apparent in smokers (4, 6, 13, 14).

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EFFECT OF EXOGENOUS ESTROGENS ON LH AND FSH SECRETION IN WOMEN WITH HYPOTHALAMIC AMENORRHEA

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Abstract The effects of estradiol on FSH and LH secretion in 5 women with hypothalamic amenorrhea are presented. The functional capacity of the pituitary to secrete FSH and LH had been proved by testing with synthetic luteinizing hormone releasing hormone (LH RH) 100 µg/m. The thyroid and adrenal functions were normal. In 3 patients estrogens were low normal and in the other 2 extreme hypoenestrogenism was found. Twelve days after the appearance of withdrawal bleeding each patient was given 10 mg estradiol benzoate (EB) i/m. In 3 patients a significant increase in the LH and a slightly smaller increase in the FSH concentration were noted with an LH peak 60 to 120 hours after injection of EB. The first of these 3 patients became pregnant in the second the biphasic 3BT curve, urine pregnandiol excretion above 2.5 mg/24 hr and the histological finding of secretory endometrium indicated that ovulation had been successfully induced while in the third patient despite a satisfactory increase in the LH and FSH concentrations ovulation could not be induced. In the remaining 2 patients no increase in the LH and FSH concentrations was noted even as late as 96 hours after the injection of EB. In these women bleeding began on the 18th and 22nd day of the cycle respectively when histological examination revealed proliferative endometrium. The first 3 cases are classified as hypothalamic amenorrhea with dysfunction of the cyclic centre and the other 2 as hypothalamic amenorrhea with dysfunction of the tonic centre. The effect of exogenous estrogens in patients with hypothalamic amenorrhea depends both on the functional capacity of the hypophysis and the capacity and condition of the tonic and cyclic centres in the hypothalamus.

INTRODUCTION

Hypothalamic amenorrhea denotes a condition associated with disorders of gonadotropin secretion due to abnormal functioning of the hypothalamus. The gonadotropic integrity of the hypophysis can be successfully tested by luteinizing hormone

releasing hormone (LH RH) (1, 2, 11-13). In addition the increase in LH and FSH concentration following the administration of LH RH is influenced significantly by the level of endogenous estrogens (2, 3, 11).

In a normal menstrual cycle there exists a dynamic but as yet insufficiently understood mechanism regulating interrelationships on the hypothalamus-pituitary-gonadal axis in which according to Leyendecker et al (6) estrogens play an important role in the preovulatory rise of LH.

The estrogen/gonadotropin ratio has been extensively studied both in the normal menstrual cycle (4, 6, 9, 16) and in some pathological conditions (5, 6, 10, 14, 15).

The aim of this study was to ascertain whether it is possible to elicit a positive feedback effect by exogenous estrogens in women with hypothalamic amenorrhea who had been tested previously with LH RH.

MATERIAL AND METHODS

Five women with secondary amenorrhea of 11 to 37 months duration were studied. Total estrogens in 24 hr urine and serum LH and FSH concentrations were at a low normal level and in 2 patients (P.C. and G.I.) low gonadotropin concentrations were accompanied by extreme hypoenestrogenism. The thyroid and adrenal functions were normal in all cases and X-ray findings did not reveal any enlargement of the sella turcica. The menarche had occurred at the usual time and the secondary sexual characteristics were well developed in all cases. All women had a problem of sterility. All hysterosalpingographic findings were normal and the spermograms of the husbands were also normal.

The most important clinical and laboratory data of our 5 patients are presented in Table I.

Table I Laboratory and clinical data of the 5 patients

Patient	Age	Duration of amenorrhea (months)	Before treatment			Previous treatments and results obtained with Clomiphene	Pregnancy	Progesterone test
			TE $\mu\text{g/l}$ 24 hr	FSH mIU/ml	LH mIU/ml			
R K	28	9	15.6	9.5	12	2 treatments 2 ovulatory responses	0	+
D C	23	14	16.4	7	19.4	2nd phase 12 days	1*	+
M L	32	26	17	6	14	2 treatments 1 ovulatory response	0	+
P C	24	17	9	4	5.1	3 treatments 1 ovulatory response	0	-
G I	26	37	7	4.2	6	2 treatments with out effect	0	-
						3 treatments with out effect	0	-

Total estrogens (TE) FSH and LH are mean values of 2 or 3 measurements

* In 1971 normal delivery with lactation in puerperium

The gonadotropic integrity of the pituitary was tested with synthetic LH RH (obtained from Hoechst Frankfurt Germany) administered in doses of 100 μg i m and the serum LH and FSH concentrations were determined according to the radioimmunoassay of Midgley (7, 8). Two to five months after this first test the first 3 patients in Table I received 4x25 mg progesterone i m and patients P C and G I 3x1 ampoules of lutealrol (20 mg progesterone and 2.5 mg estradiol benzoate) Twelve days after the appearance of withdrawal bleeding each patient was given 10 mg estradiol benzoate (EB) i m. Besides basal body temperature (BBT) serum LH and FSH concentrations were measured on the 1st 5th 10th and 11th day and between days 12 and 16 twice daily (at 7 a m and 7 p m). Pregnenediol excretion in 24 hr urine was determined on the 24th day.

Endometrium samples for histological examinations are taken on the first day of bleeding.

RESULTS

Serum LH and FSH concentrations following injection of 100 μg LH RH i m are presented in Table II.

The significant increase in the LH concentration

and the slightly smaller increase in the FSH concentration found in all patients proved the capacity of the pituitary to synthesize and secrete gonadotropins.

In patients P C and G I who showed an extreme hypoeestrogenism and a negative response to Clomiphene stimulation the increase in LH and FSH concentration following LH RH was slightly smaller. The LH and FSH concentrations obtained before and after administration of EB are presented in Figs 1 and 2.

The first 3 patients (Fig 1a b c) responded to EB with a significant increase in LH and a slightly smaller increase in FSH concentration showing LH peak 60 to 84 hr after the injection of EB.

Patients D C and M L had biphasic BBT and the 24 hr urine pregnenediol excretion on the 24th day of the cycle amounted to 3.4 and 4.6 mg respectively.

In both patients ovulation with a sufficient luteal phase was successfully induced as evidenced by the consecutive pregnancy of patient M L and the

Table II Serum LH and FSH concentrations before and after administration of 100 μg LH RH i m

Patient	LH mIU/ml			FSH mIU/ml		
	Before LH RH	Minutes after LH RH			Before LH RH	Minutes after LH RH
		30	60	120		30 60 120
R K	12	39	37	29	9.5	23 25 16
D C	19.4	56	54	38	7	21 24 11
M L	14	44	45	25	6	19 21 15
P C	5.1	16	15	8	4	9 10.5 5
G I	6	21	20	9	4.2	12 11 7

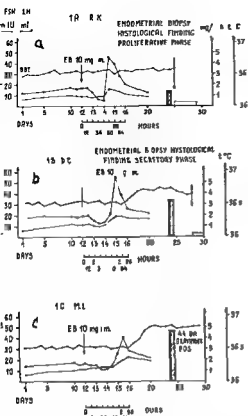


Fig 1 A B C Serum FSH and LH concentration before and after EB pregnanediol in urine mg/24 h and BBT

histological finding of secretory endometrium a biphasic BBT curve and a pregnanediol excretion of above 2.5 mg/24 hr in patient D C

In patient R K ovulation could not be induced despite an adequate increase in the LH and FSH concentrations following administration of EB. The BBT curve was monophasic urine pregnanediol excretion 1.4 mg/24 hr and bleeding began on the 15th day of the cycle when histological examination revealed proliferative endometrium. In patients B C and G I no increase in serum LH and FSH concentrations could be noted even 96 hr following the injection of EB. Their BBT curves were monophasic bleeding occurred on the 18th and 2nd day of the cycle respectively when histological examination showed proliferative endometrium.

DISCUSSION AND CONCLUSION

Thanks to the use of LH RH it is now possible to differentiate between hypothalamic and pituitary

amenorrhea. According to Gual (2) and Espinoza Campos et al (1) hypothalamic amenorrheas can be divided into two categories.

(1) Hypothalamic amenorrhea with dysfunction of the cyclic centre characterized by low gonadotropin and estrogen levels and a positive response to Clomiphene and LH RH. In these patients thanks to the intact tonic centre in the hypothalamus gonadotropin synthesis and secretion are maintained to a certain degree but cyclic variations are non-existent. According to these criteria our first 3 patients in Table I (R K, D C and M L) belong to this category of hypothalamic amenorrhea.

(2) The second category consists of hypothalamic amenorrhea with dysfunction of the tonic centre. In these patients besides low gonadotropin concentrations extreme hypo-estrogenism, negative response to Clomiphene and low or delayed response to LH RH are found. We included our patients P C and G I in the category hypothalamic amenorrhea with dysfunction of the tonic centre because in addition to low gonadotropin concentrations and extreme hypo-estrogenism they showed negative response to Clomiphene and a slow response to LH RH.

The elevation of LH and FSH concentrations following administration of LH RH depends both on the functional capacity of the pituitary and the

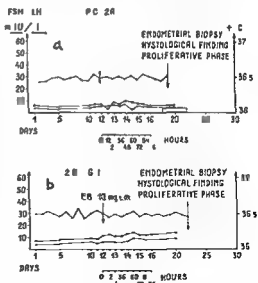


Fig 2 A B Serum FSH and LH concentration before and after oestradiol benzoate (EB) pregnanediol in urine mg/24 h and basal body temperature

amount of endogenous estrogens (11) and LH RH (13)

According to Keye & Jaffe (3) estrogens elicit the midcycle gonadotropin surge acting on the level of the hypothalamus and hypophysis

In a normal cycle the preovulatory elevation of the LH concentration is preceded by an increase in the estrogen concentration which begins 120 hr before the LH peak (2). A latent period after administration of exogenous estrogens was also noted in cases of amenorrhea. In oligo- and amenorrheic patients Taymor et al (14) and Thompson et al (15) found LH peaks 48 to 66 hr after injection of EB and Leyendecker et al (6) detected a significant increase in the LH concentration 2 days after intramuscular injection of 25 mg EB and LH peaks 4 days following the injection. In our 3 patients with a positive response the LH peak was observed 66 to 72 hr after injection of EB. In the 2 women with dysfunction of the tonic centre in the hypothalamus elevation in the LH and FSH concentrations could not be detected even as late as 96 hr after injection of EB despite the capacity of the pituitary to synthesize and secrete gonadotropins as demonstrated by the positive response to LH RH. These 2 patients with extreme hypo-estrogenism also failed to respond to Clomiphene which according to Gual (2) exerts its effects through the cyclic centre in the hypothalamus. This observation supports the hypothesis of Leyendecker et al (6) who suggests that the positive feedback effect of estrogens is also exerted via the cyclic centre. It appears however that both clomiphene and estrogens can elicit favorable effects only when the function of the tonic centre in the hypothalamus which maintains the synthesis and secretion of pituitary gonadotropins without cyclic variations is preserved. In patients with an impaired tonic centre in the hypothalamus exogenous estrogens and Clomiphene fails to take effect. In hypothalamic amenorrhea with dysfunction of the cyclic centre it is possible to elicit an increase in LH and FSH secretion by administration of exogenous estrogens and if one of the follicles is in the advanced stage of maturation rupture of the follicle and expulsion of the ovum may ensue. According to Kupperman et al (5) ovulation and pregnancy can be successfully induced (our patients D. C. and M. L.) by exogenous estrogens in women with an inadequately developed follicle which before ovulation does not secrete the necessary threefold amount of estrogen in whom the LH

peak indispensable for the rupture of the graafian follicle fails to appear. On the other hand an increase in the gonadotropin concentration after injection of EB with an adequate LH peak does not mean that ovulation has been induced (our patient R. K.). Taymor et al (14) have found that ovulation cannot be induced by the administration of exogenous hormones if the follicle has not reached a certain degree of maturity despite an adequate increase in the gonadotropin concentration and LH peak like that in a normal biphasic cycle. The effect of exogenous estrogens in hypothalamic amenorrhea does not depend on the gonadotropin integrity of the pituitary alone but also on the functional capacity of the tonic and cyclic centres in the hypothalamus. In the case of an intact tonic centre exogenous estrogens may elicit a positive feedback effect while in the case of an impairment of the tonic centre exogenous estrogens in addition to clinical and laboratory tests may be used for the differentiation of cases of hypothalamic amenorrhea into those with dysfunction of the tonic and those with dysfunction of the cyclic centre.

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α_1 FETOPROTEIN IN CORD SERUM CORRELATED TO GESTATIONAL AGE

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Abstract. Measurement of α_1 fetoprotein by rocket immunoelectrophoresis in cord serum from a consecutive series of 472 newborn infants showed a negative correlation with gestational age. The coefficient of correlation was -0.70 for girls and -0.74 for boys. For a given α_1 fetoprotein concentration in cord blood the confidence limits for prediction of gestational age for both sexes were calculated to ± 24 days. It is concluded that determination of α_1 fetoprotein in cord serum is of limited help in the evaluation of maturity.

Since 1944 α_1 fetoprotein (AFP) has been known as fetoprotein specific alpha globulin (9) and for the last 10 years as a serological marker for human hepatoma and embryonal carcinoma. The concentration of this protein in maternal serum is maximal around the 14th week of gestational age and thereafter decreases to reach low levels at term (2, 5, 6). In normal adult sera from non pregnant subjects the amount of α_1 fetoprotein is low and not measurable by immunoelectrophoresis. However, in cases of primary liver cancer and embryonal carcinoma in amniotic fluid serum α_1 fetoprotein levels reappear in adults as well as in children (1, 4). A close negative relation between the concentration of α_1 fetoprotein in cord serum from newborn infants and gestational age has been reported recently (3, 8, 9). The purpose of the present work was to study α_1 fetoprotein in cord serum obtained from a consecutive series of newborn infants of varying gestational age.

MATERIAL

Samples of cord blood were collected just after delivery as a consecutive series over a 6-month period. After coagulation the blood samples were centrifuged and the

sera were kept frozen at -18°C until determination of the α_1 fetoprotein concentration. The maximal time interval between collection of samples and analysis was 3 days.

With the intention of obtaining the most precise information about gestational age the following criteria had to be fulfilled:

Information on the exact date of the last menstrual period should be available. No bleeding should have occurred during the first trimester.

Pathological pregnancies with toxæmia, immunisation or malformation were not included in the series. Twins were not included either. The birth weight of the infants was recorded by the midwife just after delivery.

METHOD

α_1 fetoprotein was determined by quantitative rocket immunoelectrophoresis as described by Laurell (7).

Apparatus. Complete equipment for immunoelectrophoresis (Dansk Laboratørudstyr, Copenhagen).

Standard. α_1 fetoprotein Standard 250 mg/l (Behringwerke, Marburg).

Antibody. Rabbit immunoglobulins to human α_1 fetoprotein (Dakopatts, Copenhagen).

Immunoelectrophoresis was carried out in a 1% agarose gel (Litex, Glostrup) in barbitone buffer pH 8.6 containing antibody 0.7% (V/v) with 2 V/cm for 12-18 hours. Standards were diluted with barbitone buffer (V/v) 1+3, 1+7, 1+15 and 1+31 and sera were diluted up to

Table I. The coefficients of correlation (r) between α_1 fetoprotein in cord serum, gestational age and birth weight.

Correlation	♂	♀
AFP~gest age	$r = -0.74$	$r = -0.70$
Weight~gest age	$r = 0.69$	$r = 0.56$
AFP~weight	$r = -0.62$	$r = -0.57$

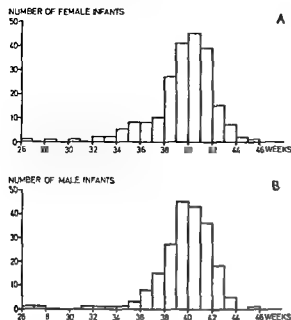


Fig 1 The cases studied grouped according to sex and gestational age

1+9 prior to analysis of 5 μ l. After washing the plates were dried and stained with coomassie brilliant blue. By this method the lower limit of determination of α_1 fetoprotein is about 5 mg/l. Day to-day variation was 6% as calculated from 10 replicate determinations on a control serum.

Statistical method

Gestational age and birth weight was correlated with α_1 fetoprotein concentration using linear regression. This is

permissible because for both sexes the scatter of gestational age points around the regression line is dependent of whether the gestational age is normal or very low. The same argument applies to the birth weight. The slopes and the intercepts of the regression lines were calculated as well as the correlation coefficients, the standard deviations for the position of the regression lines and the 95% confidence limits for estimated gestational age.

RESULTS

Only 422 out of 600 cases fulfilled the above-mentioned criteria. The distribution according to gestational age for 207 girls is illustrated in fig. 1A and for 215 boys in fig. 1B. As seen from the figures 9 girls and 19 boys were born before the 36th week of gestation. The majority of the children were at term, i.e. with gestational age >260 days.

Table I shows the correlations between the concentration of α_1 fetoprotein in cord serum, the gestational age and the birth weight. The coefficient of correlation (r) for α_1 fetoprotein versus gestational age was -0.70 and -0.74 for girls and boys respectively. This was a slightly higher degree of correlation than for birth weight versus gestational age. The correlation between α_1 fetoprotein and birth weight was less pronounced: $r=0.57$ and $r=0.61$ for girls and boys respectively. However all correlation coefficients are highly significant.

Fig. 2 shows the correlation between the concentration of α_1 fetoprotein (AFP) in mg/l and gestational age as evaluated by linear regression.

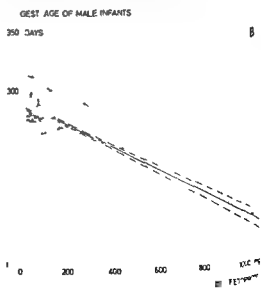
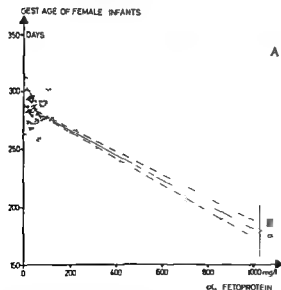


Fig 2 The correlation between α_1 fetoprotein mg/l and gestational age for female (A) and male infants (B). The

dot and dash lines indicate the uncertainty for the regression lines and the dotted lines the 95% confidence limits.

Analysis For 207 girls (Fig 2A) the following equation was calculated Days of gestation = $-0.107 \times \text{AFP} + 288$ The 95% confidence limits were ± 23 days

For 215 boys (Fig 2B) Days of gestation = $100 \times \text{AFP} + 290$ The 95% confidence limits were ± 25 days α_1 fetoprotein = AFP in mg/l For both sexes taken together the 95% confidence limits for determination of the gestational age were ± 24 days

For 47 preterm infants (gestational age < 260 days) we found a correlation coefficient $r = -0.81$ between α_1 fetoprotein and gestational age

DISCUSSION

The results of the present investigation showed that the concentration of AFP in cord serum from newborn infants is correlated with gestational age although it is not as sound an indication of maturity as stated by some authors (6-8). The calculated 95% confidence limit for determining gestational age by means of the α_1 fetoprotein concentration in cord serum was ± 24 days. This corresponds to the value found by Bergstrand et al. (3) who reported limits of ± 4.3 weeks. In the work of Nørgaard-Pedersen (8) the limit was ± 16 days. This difference might be explained by differences in the selection and character of the cases studied. In our series infants with low gestational age were less frequent represented than in the series of Nørgaard-Pedersen (8). According to Nørgaard-Pedersen the correlation between the concentration of α_1 fetoprotein and gestational age is better for preterm infants than for children with a gestational age over 280 days. Similarly we found a coefficient of correlation of 0.81 in preterm infants.

The conclusion of our study is that determination of α_1 fetoprotein in cord serum is hardly a valuable

supplement to external characteristics e.g. birth weight in the evaluation of maturity.

However the number of preterm infants in our series is limited so that further investigations will be necessary at this point.

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THE SIGNIFICANCE OF HUMAN CHORIONIC GONADOTROPIN IN BLOOD SERUM FOR THE EARLY DIAGNOSIS OF ECTOPIC PREGNANCY

A. Milwidsky, A. Adoni, Z. Paltu, M. Stark and S. Segal

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Abstract Determination of human chorionic gonadotropin (HCG) values in the serum by the radioimmunoassay technique was performed in 23 women with suspected ectopic pregnancies. In 16 cases the values of HCG were high and the diagnosis of ectopic pregnancy was verified by laparoscopy and laparotomy. In 7 cases low HCG values were found and ectopic pregnancy was excluded. The detection of HCG in the serum was found to be an excellent tool for the early diagnosis of ectopic pregnancy, helping to prevent the dangerous sequelae which follow the late diagnosis of this condition.

Early detection of an ectopic pregnancy is difficult as much as there is no simple laboratory test that will establish the diagnosis. The presence of an ectopic pregnancy can only be proved by surgery. It is a basic problem to differentiate between ectopic pregnancy and other less dangerous situations such as persistent corpus luteum, follicular cyst, imminent abortion and inflammatory adnexal diseases.

Among the laboratory methods used to diagnose ectopic pregnancy is the immunologic pregnancy test of the urine. The sensitivity of this test is 700 IU/l (1, 2). Jeffcoate (3) claims that this test is positive in only 50% of all ectopic pregnancy cases. According to others the accuracy of the test is between 76-87.5% (4, 5, 6). This means that the diagnosis of a very early or a pathologic pregnancy could be missed using this method.

The purpose of this study was to evaluate the radioimmunoassay technique of HCG in the serum for the early diagnosis of ectopic pregnancy and to try to eliminate the need for surgery in many suspected cases.

MATERIAL AND METHODS

Twenty three women were admitted to our hospital during a 12 month period (1974-5) with suspected ectopic pregnancy. All (except the last 3 in the control group) underwent the routine procedure of examination under anaesthesia and laparoscopy. In all cases a laparotomy was performed when the diagnosis was established. On admission 10 ml of venous blood was drawn, the serum was separated and was later sent for determination of HCG values by the double antibody radioimmunoassay technique (7). (The radioimmunoassay kit was supplied by Sorinco-Italy.)

RESULTS

Fourteen of the 23 women had ectopic pregnancies as proven by laparotomy and histology (Table I). The 9 patients where no ectopic pregnancies were found served as a control group (Table II).

In all cases of ectopic pregnancy HCG values in the serum were high. They ranged from 182-884 mIU/ml.

In the control group the range of HCG was 9.1-56.0 mIU/ml. Immunologic urine tests for pregnancy were performed in only 9 out of the 14 cases with the ectopic pregnancy. Five gave negative and 4 gave positive results. In the control group all cases showed negative results.

DISCUSSION

An early diagnostic test for ectopic pregnancy would help to decrease the morbidity and mortality rate. In many cases it could change the prognosis for future pregnancies.

The urine immunological pregnancy test is not satisfactory for early diagnosis of ectopic pregnancy because of its low limit of sensitivity. A negative result does not exclude trophoblastic activity (8).

Table I Values of HCG in blood serum in cases of ectopic pregnancy

Patient No	Age	Week of pregnancy	Laparo-scopy	Laparo-tomy	HCG (mIU/ml)	HIT
1	30	11	+	+	884	Negative
2	30	7	+	+	611	-
3	25	7	+	+	442	Negative
4	22	7	+	+	806	-
5	24	7	+	+	182	-
6	31	9	+	+	80.6 ^a	Negative
7	26	8	+	+	598	Positive
8	28	6	+	+	299	Positive
9	24	8	+	+	702	Positive
10	35	6	+	+	728	-
11	24	6	+	+	364	Positive
12	32	6	+	+	552	Negative
13	34	8	+	+	325	-
14	24	6	+	+	858	Negative

Haemagglutination Inhibition Test

^a In this case the blood was taken two days after the operation

Barjaktarovic (9) in 1934 showed that the placenta in cases of ectopic pregnancy secretes less HCG than a placenta of a normal intrauterine pregnancy of the same gestational age. This was later confirmed by others (10, 11).

The radioimmunoassay of HCG in the serum offers a much more sensitive test for measuring trophoblastic hormones in the serum than the urine test.

In all 14 cases where a high level of HCG was found an ectopic pregnancy was proved by laparoscopy and laparotomy. In 6 of the patients where HCG levels were low laparoscopy excluded the possibility of an ectopic pregnancy. In the last 3 low values of HCG were measured and laparoscopy was not performed. These patients were carefully observed till normal periods resumed a few weeks later.

Early diagnosis of ectopic pregnancy may have

great significance for the reproductive future of a woman. It would enable an early surgical procedure which might prevent tubal rupture with its associated haemorrhage and shock. A conservative plasty sometimes may be the procedure of choice.

For the non pregnant woman having symptoms similar to an ectopic pregnancy the low HCG levels determined using the radioimmunoassay test may avoid the need for general anaesthesia, the operative procedure of laparoscopy and curettage and save hospital time.

Luteinizing hormone (LH) and HCG have immunologic cross reaction in this method. The values of HCG+LH measured in all 14 cases of ectopic pregnancy were higher than 150 mIU/ml. In non pregnant woman the highest value was 37.5 mIU/ml. We feel that a value of 150 mIU/ml may serve as the diagnostic limit between a pregnant and non pregnant woman. However it is possible

Table II Values of HCG in blood serum in cases without ectopic pregnancy

Patient No	Age	Laparoscopy	Laparotomy	HCG (mIU/ml)	HIT
1	33	+	-	9.1	Negative
2	24	+	-	22.1	Negative
3	27	+	-	7.0	Negative
4	38	+	-	22.0	Negative
5	32	+	-	13.0	Negative
6	30	+	-	37.5	Negative
7	25	-	-	43.0	Negative
8	25	-	-	49.0	Negative
9	30	-	-	56.0	Negative

very rare cases one may find high levels of LH during the mid cycle surge in normal menstruating men

This problem can be resolved by using a test which differentiates between HCG and LH. The differentiation is performed by measuring the HCG subunit. Although this test is somewhat longer it provides the physician with a more positive diagnostic procedure.

In conclusion it seems that the measurement of HCG in the serum by the radioimmunoassay technique is an important tool in the early diagnosis of ectopic pregnancy.

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PNEUMOPERITONEUM WITHOUT CLINICAL PERITONITIS DUE TO BILATERAL PYOSALPINX

Odd Sørensen, Willy Haukeland and Tormod Bjerkeset

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University Hospital of Bergen, Bergen, Norway*

Abstract A case of pneumoperitoneum without clinical peritonitis due to bilateral pyosalpinx is presented. This unusual condition has in the literature been called spontaneous or idiopathic pneumoperitoneum, and no operative treatment in such cases has been advocated. The patient was a 45-year-old woman who had been operated on for bilateral salpingitis 10 years before admission. She was admitted to hospital because of abdominal pain and distension. On admission it was noted that her abdomen was moderately distended but no signs of peritonitis. Temperature 38.0°C, Hb 14.3 g%, WBC 23,600 per mm³, Sedimentation rate 48 mm/hour. Blood glucose 3.9 mg%. The patient's diabetes mellitus was treated with insulin.

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Abstract A case of pneumoperitoneum without clinical peritonitis due to bilateral pyosalpinx is presented. This clinical condition has in the literature been called spontaneous or idiopathic pneumoperitoneum and conservative treatment in such cases has been advocated. In our case severe intraperitoneal pathology may exist and we therefore advocate early explorative laparotomy in all patients with pneumoperitoneum without peritonitis.

The term pneumoperitoneum is used to describe the presence of free gas in the peritoneal cavity. In most patients pneumoperitoneum is considered as evidence of an abdominal catastrophe and is usually accompanied by signs of peritoneal irritation or peritonitis (6). In such cases immediate surgical intervention is mandatory.

An etiological classification of pneumoperitoneum has been presented by Loughhead (10). Fig 1 shows a modification of his classification system with reference to the actual literature.

Pneumoperitoneum due to intraperitoneal infection caused by gas producing organisms of non-ostrioidal origin has been reported by other authors.

14) Recently one case was encountered with pneumoperitoneum without clinical peritonitis. At surgery bilateral pyosalpinx was found. No similar cases of this nature were found in the literature. As new cause is added to the list for differential diagnosis of pneumoperitoneum it is felt that a report is indicated.

CASE REPORT

On a woman aged 73 was admitted to the medical department Sept 18th 1973 with the diagnosis of diabetic coma. She had previously been healthy except the last 4 months when polydipsia, polyphagia and polyuria

were experienced. On admission it was noted that her abdomen was moderately distended but no signs of peritonitis. Temperature 38.0°C Hb 14.3 g% WBC 23,600 per mm³. Sedimentation rate 48 mm/hour. Blood glucose 344 mg%. The patient's diabetes mellitus was treated with insulin.

During her stay in the medical department it was noted that the abdominal distension increased. The possibility of ileus/subileus was discussed and an X ray of the colon was performed Sept 27. During this examination which showed displacement of the rectum possibly due to a pelvic tumour massive pneumoperitoneum was found (Fig 2).

A careful physical examination was again performed. The patient had no abdominal pain or no signs of peritonitis. A check plain X ray of her abdomen disclosed increasing pneumoperitoneum and the patient was admitted to the surgical department. Physical examination showed an afebrile woman. The abdomen was distended and there were normal bowel sounds. No abdominal tumor could be found. Hb 14.2 g% WBC 13,800 per mm³. Sedimentation rate 31 mm/hour.

On Oct 8 laparotomy revealed bilateral pyosalpinx and a salpingo-oophorectomy was performed. Bacteriological study of the pus showed *E. coli*, *Proteus mirabilis* and non-hemolytic streptococcus. No anaerobic bacteria could be demonstrated. Postoperative plain X ray of the abdomen showed disappearance of the pneumoperitoneum.

DISCUSSION

Pneumoperitoneum without clinical peritonitis is a rare experience in surgical practice. This clinical condition has in the literature been called spontaneous or idiopathic pneumoperitoneum (1, 7, 11, 16) and many authors advocate conservative management and close observation in asymptomatic pneumoperitoneum of undetermined origin (3, 13, 19).

As demonstrated in our case intra abdominal

ETIOLOGY OF PNEUMOPERITONEUM			LITERATURE
MECHANICAL	DIAGNOSTIC AND THERAPEUTIC PROCEDURES	POSTOPERATIVE	Bu J Rad 41 57 515 1962
		THERAPEUTIC AND DIAGNOSTIC	Carr h V Shine Glover and V'hinger Rosenfeld papers
		LAP ROCENTESIS	Quang Th am Vo Ing Bull 1977
	WOUNDS	FOLLOWING TEST FOR PATENCY OF FALLOPIAN TUBES	Ac h ing
		EXTERNAL	B J Rad 118 290 19 5
		ABDOMINAL WALL INCLUDING PERITONEUM	Cable W Surge p 2
		RE FORATION BY RECTAL THERMOMETERS ENEMA TIP ETC	Fish Ed La l Ltd London 196
		AIR INSUFFLATION GASTROSCOPE	Quang Th am 2 8 3 5
		AIR INSUFFLATION PHOTOSCOPE / SIGMOIDOSCOPE	Ar h S ing 42 850 1941
		AIR CONTRAST BARIUM ENEMA	Quang Th am 15 258 1975
		VAGINAL DOUCHING	A h S ing 38 500 1939
	INTERNAL	PNEUMOTHORAX ACCIDENTAL PUNCTURE	Quang Th am 38 620 1945
		PNEUMOTHORAX THROUGH TRACHEAL OPENINGS FISTULA	Quang Th am 196
SPONTANEOUS	PERFORATION OF VISCUS		Am J Surg 113 567 196
	DUE TO UNKNOWN CAUSE		Am J Surg 101 232 1 1
	STERILE PERFORATION OF THE COLON		A h S ing 100 61 1970
FALSE	HEPATIC FLEXURE OF COLON BETWEEN LIVER AND DIAPHRAGM (CHILADITIS SYNDROME)		Fleming Dahl J Rosenfeld
	TRANSPOSITION OF VISCERA WITH STOMACH AIR		Quang Th am 38 620 1945
	BUBBLE UNDER RIGHT DIAPHRAGM		Quang Th am 38 620 1945
OTHERS	SUBPULMONARY AIR		Quang Th am 38 620 1945
	AEROPHAGIA		Cl 1 and Cl 2 Qu 37 149 197
	PNEUMATOSIS CYSTOIDES INTESTINALIS		J Trauma 16 433 1976
	PREEXISTINGLY PLACED INTUBATION TUBES DURING ANESTHESIA PROCEDURES		Am J g on 22 523 1976

Fig 1 Etiological classification of pneumoperitoneum with reference to literature

pathology may exist without clinical symptoms in patients where pneumoperitoneum is found. Peritonitis with pneumoperitoneum caused by bacteria of non clostridial origin has been demon-

strated (14). The gas in these cases has been caused by Gram negative intestinal bacteria of which coli is well known to produce gas due to fermentation of carbohydrates (2, 14).



Fig 2 Plain abdominal X ray demonstrating free intraperitoneal gas

the bacteriology of intra peritoneal infections recently been studied (18). It was shown that cultures commonly contained many different bacteria. Anaerobic bacteria were isolated most frequently. Many of these anaerobic bacteria as well as facultative anaerobic micro-organisms may be gas producing (2, 8).

Spontaneous or idiopathic pneumopentoneum is discussed in detail by other authors (3, 5, 12). We believe that this term is perhaps a misnomer. Many authors use the term in cases where clear intra abdominal pathology is demonstrated (7, 9, 11). The words spontaneous or idiopathic in these cases are not descriptive but are used to stress the fact that no signs of intra abdominal pathology could be demonstrated prior to operation. Only few cases of idiopathic pneumopentoneum are found in modern literature (13, 19). The important fact is that pneumopentoneum is a sign of grave intra abdominal pathology as in steroid perforations of the colon where the clinical signs are vague (17).

Concerning treatment of pneumopentoneum without peritonitis we completely disagree with authors who advocate conservative management. We feel that excluding intraperitoneal pathology in a patient with pneumopentoneum without clinical symptoms is difficult. A high grade of suspicion is mandatory in such cases and an explorative laparotomy could be performed in all patients.

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STUDIES IN CHOLESTASIS OF PREGNANCY

VI Fatty Acid Composition of Glycero-phospholipids before and after Delivery

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Abstract Eight pregnant women complaining of generalized pruritus with lipoprotein X (LP X) in their serum and diagnosed as cases of cholestasis of pregnancy (CP) were studied during pregnancy and after delivery. Ten women with uncomplicated normal pregnancy served as controls. LP X, liver function tests and relative fatty acid composition of serum lecithin (determined by gas-liquid chromatography (GLC)) were followed. The fatty acid composition in liver and serum lecithin is determined by the synthesis pathways of lecithin in the liver. The major and quantitatively dominating cytidine-diphosphate dependent pathway, pathway I, causes the appearance of lecithin with palmitic acid (16:0) in 1 position and oleic (18:1) or linoleic (18:2) acid in 2 position, while pathway II, with methylation of phosphatidyl-ethanolamine (cephalin), preferentially causes the appearance of lecithin with stearic acid (18:0) in 1 position and arachidonic acid (20:4) in 2 position. Pathway I is enhanced by oestrogenic influence, while pathway II is inhibited. During pregnancy women with CP were characterized in their serum lecithin fatty acid composition by a high palmitic (16:0) and a high oleic (18:1) acid content in agreement with our earlier studies. After delivery in women with prior CP there was a decrease in palmitic (16:0) and linoleic (18:2) acids and an increase in stearic (18:0) acid, which was interpreted as decreased influence on the major lecithin synthesis pathway and an enhancement of pathway II. In addition, after delivery in the lactating mother, serum lecithin fatty acid composition data revealed an essential fatty acid (EFA) deficiency. It was earlier shown that women with prior CP (when studied 8-21 months after delivery) had a basic metabolic defect, expressing presumably decreased estrogen enhanced pathway II of liver lecithin synthesis. In the present study, soon after delivery (on day 4-8), women with prior CP showed, however, less pathway II deficiency than women with a prior normal pregnancy. This was interpreted as a persistence of the cholestatic influence on liver lecithin synthesis pathways III this short time after delivery. Serum lecithin fatty acid composition appears to be a sensitive variable for the evaluation of cholestatic influences in the liver.

Lipid metabolism is altered in pregnant women with cholestasis, i.e. cholestasis of pregnancy (CP) as compared to normal pregnant women (6, 8, 13). The cholestatic pregnancy, as other cholestatic conditions, is accompanied by the occurrence in serum of an abnormal lipoprotein, lipoprotein X (LP X), that might at least partially explain the increased serum lipid levels. The serum concentrations of cholesterol, triglycerides and phospholipids are higher in CP and reach a maximum level just before delivery (8, 13). After delivery, all symptoms and signs of CP subside rapidly and laboratory data usually return to normal within two or three weeks after delivery (7).

The aim of the present study was to follow the disappearance of LP X and the characteristic fatty acid pattern of serum lecithin before and a few days after delivery in women with CP.

MATERIALS AND METHODS

Clinical series Eight women (mean age 25.7, range 21-29 years) with generalized pruritus but with otherwise uncomplicated pregnancy were studied. Blood samples were drawn during pregnancy (weeks of gestation: mean 39.9, range 39-41) and on day 4 to day 8 after delivery.

Control series Ten women (mean age 24.2, range 20-28 years) without symptoms or signs of pruritus and with otherwise normal pregnancy served as control series. Blood samples were drawn during pregnancy (weeks of gestation: mean 40.2, range 39-40) and on day 4 to day 8 after delivery.

Blood samples were drawn in the fasting state in the morning. Blood was allowed to clot for 30 minutes at room temperature and was then centrifuged at 2500 r.p.m. for 20 minutes. Serum specimens for gas-liquid chro-

matography (GLC) analyses were immediately frozen and stored at -20°C in glass tubes with teflon screw caps.

Liver function test, total serum bilirubin (normal <1.2 mg/100 ml) Alkaline phosphatase (normal <260 units/l) SGOT (ASAT) (normal <17 units/l) and SGPT (ALAT) (normal <17 units/l) were determined at the Laboratory of Clinical Chemistry according to standard methods.

Lipoprotein X (LP X). Semi-quantification of LP X was performed by double immunodiffusion in 1% agar gel employing barbital buffer pH 8.6 ionic strength 0.05. Rabbit serum containing antibodies to human LP X was obtained through the courtesy of Dr D. Seidel Heidelberg West-Germany. All plates were kept in a moist chamber at room temperature and the immunoprecipitation lines were visually evaluated. For details in methods cf. (7).

Gas liquid chromatography (GLC) procedure. Preparation of lipid extracts: separation of lipids by thin-layer chromatography on Silica-gel and isolation of lecithin and phosphoglyceride spots and preparation of fatty acid methyl esters were performed as described earlier (6). GLC of methyl esters: measurement of serum lecithin and lecithin fatty acids and conversion from weight per cent to mole per cent have been described in an earlier publication (6).

Statistical methods. Conventional methods were used for the calculation of means, standard deviations and standard error of means. Student's *t* test was used to study differences between groups. Values of $p \geq 0.05$ were considered statistically significant (2).

RESULTS

Maternal data. In the series of women with CP no maternal complications occurred during delivery. Maternal and infant birth weights were within expected limits. After delivery day 4–day 8 at the time of blood sampling none of the women revealed pruritus and all were lactating. Identical data were true for the control series.

Liver function tests. After delivery at day 4–day 8 liver function tests were within normal limits in the series of women with CP. Mean SGOT and SGPT were reduced as compared to during pregnancy.

Lipoprotein X (LP X). During pregnancy all women with CP revealed LP X in serum. After delivery already at the first examination on day 4–day 8 LP X had disappeared in all women with prior CP.

Fatty acid composition of serum glycerophospholipids. In women with CP the fatty acid composition of glycerophospholipids (GPL) showed identical changes with those of lecithin (PC).

Table I Relative composition of major fatty acids of serum lecithin and mean differences (Δ) in women with cholestasis of pregnancy ($n=8$ weeks of gestation mean 39 weeks range 39–41) and in women 4–8 days after delivery.

Figures are given in mole per cent of methyl ester level. * 0.01 level.

Cholestasis of pregnancy			
	During pregnancy ($\bar{x} \pm \text{S.E.M.}$)	4–8 days after delivery ($\bar{x} \pm \text{S.E.M.}$)	Non-pregnant Δ
16:0	35.0 \pm 0.72	33.0 \pm 0.73	–2.0
18:0	10.0 \pm 0.94	11.9 \pm 0.90	+1.9
18:1 ($n=9$)	13.9 \pm 0.56	15.1 \pm 0.51	+1.2*
18:2 ($n=6$)	27.2 \pm 0.98	24.6 \pm 1.14	–2.6
20:3 ($n=6$)	3.2 \pm 0.16	3.4 \pm 0.18	+0.2
20:4 ($n=6$)	5.2 \pm 0.36	5.8 \pm 0.37	+0.6
22:6 ($n=3$)	2.7 \pm 0.31	2.6 \pm 0.38	–0.1
18–22 ($n=6$)	37.8 \pm 0.62	34.2 \pm 0.75	–3.6
Total serum lecithin (mg/100 ml)	258 \pm 14.5	194 \pm 16.8	–64

Fatty acid composition of serum lecithin (PC) I. After delivery, as compared to during pregnancy (week 39–41) in the series of women with CP (Table I) the mean fatty acid composition showed

Table II Relative composition of major fatty acids of serum lecithin and mean differences (Δ) in normal pregnant women ($n=10$ weeks of gestation mean 40.2 weeks range 39–41) and in same women 4–8 days after delivery.

Figures are given in mole per cent of methyl ester level. * 0.01 level.

Normal pregnancy			
	During pregnancy ($\bar{x} \pm \text{S.E.M.}$)	4–8 days after delivery ($\bar{x} \pm \text{S.E.M.}$)	Non-pregnant Δ
16:0	35.1 \pm 0.53	32.2 \pm 0.4	–2.9
18:0	10.3 \pm 0.24	11.0 \pm 0.29	+0.7
18:1 ($n=9$)	12.3 \pm 0.28	13.6 \pm 0.63	+1.3
18:2 ($n=6$)	22.6 \pm 0.72	24.1 \pm 0.75	+1.5
20:3 ($n=6$)	3.5 \pm 0.13	3.1 \pm 0.18	–0.4
20:4 ($n=6$)	6.9 \pm 0.61	7.8 \pm 0.45	+0.9
22:6 ($n=3$)	5.2 \pm 0.35	4.3 \pm 0.28	–0.9
18–22 ($n=6$)	33.8 \pm 0.85	35.7 \pm 0.60	+1.9
Total serum lecithin (mg/100 ml)	245 \pm 39.0	216 \pm 9.4	–29

Table III Mean differences (Δ) (mole per cent) in composition of major fatty acids of serum lecithin in women with cholestasis of pregnancy (CP) ($n=8$) compared to normal pregnant women ($n=10$) during pregnancy (weeks of gestation mean 40) and 4-8 days after delivery

	* 0.01 level		0.001 level	
	During pregnancy CP vs normal		4-8 days after delivery CP vs normal	
	Δ	P	Δ	P
	-0.1	-	+0.8	-
	-0.3	-	+0.9	-
($n=9$)	+1.6	-	+1.5	-
($n=6$)	+4.6	-	+0.3	-
($n=6$)	-0.3	-	+0.3	-
($n=6$)	-1.7	-	-2.0	-
($n=3$)	-2.5	-	-1.7	-
($n=6$)	+4.0	-	-1.5	-
serum lecithin (100 ml)	+13	-	-27	-

Following changes after delivery: Palmitic acid (16:0) ($p<0.05$), linoleic acid (18:2) ($p<0.01$) and sum of $n-6$ ($p<0.01$) were lower and stearic acid (18:0) ($p<0.05$) and oleic acid (18:1) ($p<0.05$) were higher after delivery. Also calculated concentration of serum lecithin was lower ($p<0.01$) in the control series (Table II) the mean changes in fatty acid composition of lecithin after delivery were characterized by lower palmitic acid (16:0) ($p<0.01$) and 22:6 ($p<0.05$) and higher stearic acid (18:0) ($p<0.05$).

CP as compared to normal pregnancy during pregnancy (Table III). The mean fatty acid composition of serum lecithin in women with CP was characterized by a lower content of arachidonic acid (20:4) ($p<0.05$) and of 22:6 ($p<0.001$) and a higher content of oleic acid (18:1) ($p<0.05$) of linoleic acid (18:2) ($p<0.01$) and of sum of $n-6$ ($p<0.01$).

CP as compared to normal pregnancy after delivery (Table III). The mean fatty acid composition of serum lecithin in women with prior CP was higher in arachidonic acid (20:4) ($p<0.01$) and in 22:6 ($p<0.001$) than in the control series.

DISCUSSION

Lecithin composes 69% of serum phospholipids. Lecithin transported by serum lipoproteins originates preferentially in the liver and is there synthesized along different intracellular pathways. The faster and quantitatively most important pathway is cytidine-diphosphate:choline diglyceride pathway (pathway I) which preferably gives palmitic acid (16:0) in 1 position and linoleic (18:2) or oleic (18:1) acid in 2 position. This pathway for the synthesis of lecithin in the liver is stimulated by the presence of bile acids (3). The second important pathway (pathway II) cytidine-diphosphate:ethanolamine methylation pathway causes the appearance of lecithin containing stearic acid (18:0) in 1 position and arachidonic acid (20:4) as well as other polyunsaturated fatty acids of longer chain length and greater unsaturation e.g. 22:6 in 2 position. Pathway II is more active in the female and during the influence of estrogens (1, 4).

After delivery as compared to during pregnancy women with cholestasis of pregnancy (CP) revealed two patterns of changes in their relative fatty acid composition of serum lecithin. Firstly evidence for a decrease in pathway I simultaneously with an increase in pathway II characterized by a decrease in palmitic and linoleic acids and an increase in stearic acid. Secondly the fatty acid changes may express a relative deficiency of essential fatty acids (EFA) with a decrease in sum of $n-6$ with a compensatory increase in non-essential oleic acid.

Also in normal pregnancy changes after delivery in serum lecithin fatty acid composition indicated a decrease in pathway I and simultaneously an increase in pathway II as judged from inverse variations in palmitic and stearic acids. This in turn would suggest that the normal pregnancy as such is cholestatic (12). An alternate explanation would be that the characteristically high serum lecithin in the pregnant state would require an increased lecithin synthesis mediated presumably by the major pathway I (11).

In an earlier study (10) women with previous CP (studied 8-21 months after delivery) revealed as judged by their serum lecithin fatty acid composition a basic metabolic defect. This basic defect was characterized by increased stearic and arachidonic acids due possibly to an estrogen enhanced pathway II. In the present study after delivery (day 4-day 8) women with a prior CP showed a lower content of arachidonic acid and 22:6 than women with a prior normal pregnancy. This might indicate that the change after delivery towards an

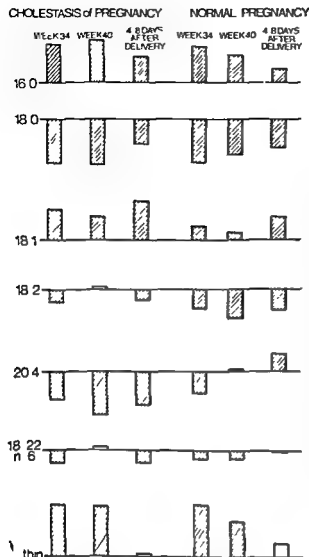


Fig 1 Changes in relative fatty acid composition of serum lecithin. Changes (expressed as per cent) between non pregnant state and gestational week 34 and 40 and 4-8 days after delivery in women with cholestasis of pregnancy (CP) and in normal pregnant women. Values for the non pregnant state (from series of previous CP 8-21 months after delivery (10)) and of normal non pregnant women (11) respectively. Shaded areas indicate significant changes.

estrogenic influence on pathway II was retarded in women with prior CP by a remaining influence from the cholestasis. The present method for evaluating metabolic influences in the liver by changes in serum lecithin fatty acid composition appears from these data to be more sensitive than those commonly used (i.e. liver function tests and LPX identification). The disappearance of

cholestasis after delivery apparent from serum lecithin fatty acid composition data (Fig. 1) the reverse pattern to that seen in the development of CP. Through the steps of pruritus gravidarum (PG) of short and long duration into hepatopregnancy (HP) serum lecithin fatty acid composition revealed initially an enhanced pathway II turning into a cholestatic pathway I influence (9).

A lower level of essential fatty acids (EFA) and an increase in oleic acid after delivery in women with prior CP is intriguing (Fig. 1). It could be expected that women with CP might suffer from latent malabsorption of EFA due to a reduced release of bile acids in the intestine. However, the present study reveals no obvious lack of EFA during pregnancy. Breast milk from the mother has a high content of linoleic acid (3.5% of total calories) to make up for low EFA in the newborn baby (9). Thus after delivery the milk produced would be expected to drain EFA from the mother, a lactating mother with a 3 kg baby would lose >1 g as linoleic acid per day. In the presence of a latent EFA deficiency during pregnancy a true deficiency in EFA could possibly be apparent during lactation. Because of this EFA deficiency should be looked for in mother and child after delivery, particularly after a cholestatic pregnancy.

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ULTRASONIC ASSESSMENT OF FETAL GROWTH

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Abstract Consecutive ultrasonic measurement of fetal biparietal diameter (B P D) and fetal chest area was carried out in 152 normal and 59 complicated pregnancies between 25 and 40 weeks of gestation. In normal pregnancy the mean fetal head area (B P D²) varied from 50 cm² at 25 weeks to 98.0 cm² at 40 weeks. The mean chest area varied from 31.3 cm² at 25 weeks to 94.3 cm² at 40 weeks. A normal curve of the head-to-chest relationship was constructed. In the group of complicated pregnancies two types of fetal growth retardation could be recognized: the first type was characterized by a normal head-to-chest relationship (symmetric type); the second type was characterized by an abnormal head-to-chest relationship (asymmetric type, relatively large head area). During fetal growth acceleration head-to-chest relationship was abnormal (relatively large chest area).

INTRODUCTION

According to Campbell (1, 2) measurement of the biparietal diameter (B P D) is the most precise fetal measurement that can be obtained antenatally. In recent years however increasing attention has been paid to ultrasonic measurements of the fetal chest (3, 4, 11, 17) and upper abdominal region (4, 5) in relation to fetal growth retardation and acceleration.

The objective of the present study was to assess whether antenatal measurement of fetal chest size could provide information complementary to that of cephalometry.

The results are presented in two parts. In the first part fetal head and chest size in relation to fetal maturity during normal pregnancy is established. In the second part the significance of serial measurements of fetal head and chest size during complicated pregnancy is demonstrated.

METHODS

1. Ultrasonic measurements were performed on the ultrasonograph 4102. Fetal head size was assessed by

means of measurement of the biparietal diameter (B P D) in mm according to Campbell's method (1). For the measurement of the fetal B P D a sound velocity of 1600 m per second was used. According to Willocks (70) this velocity is the optimum figure for assessing the true head size. As sound velocity in soft tissues is about 1540 m per second, this velocity was applied for our fetal chest size measurements. First a longitudinal B mode Ultrasonogram of a sagittal section of the foetus was obtained. The fetal spine was then located. Subsequently the transverse section at right angles to the fetal spine immediately caudal to the fetal heart pulsations was obtained and could be demonstrated on a transverse B mode Ultrasonogram (Fig. 1). At this level a section of the fetal liver can always be visualized. A polaroid photograph was made and measurement of the fetal chest area (in cm²) at this level was carried out by means of a planimeter.

In 3 patients ten ultrasonic B P D—and in another 3 patients nine ultrasonic chest recordings were performed within 24 hours on each foetus (Table I). In about 95% of the cases an individual measurement of the B P D fell within ± 1.3 mm ($2 \times S D$) and of the fetal chest area



Fig. 1 Ultrasonogram showing transverse section of the fetal chest area immediately caudal to the fetal heart pulsations. S=fetal spine, A=aorta, L=fetal liver.

Table I Evaluation of the reproducibility of fetal B P D and chest area measurement

\bar{x} = mean value S D = standard deviation

Case no	\bar{x}	S D	n	
Fetal biparietal diameter (B P D in mm)				
A	90.0	0.37	10	
B	78.3	0.94	10	
C	99.9	0.66	10	Mean S D = 0.70
Fetal chest area (cm²)				
A	63.91	0.925	9	
B	33.99	0.708	9	
C	34.10	0.875	9	Mean S D = 0.841

within ± 1.7 cm² (\approx S D) of the expected mean indicating a high degree of reproducibility.

Patients

Two hundred and eleven patients were studied. In 152 cases pregnancy was normal and in 59 cases complicated. Each patient was certain of the date of her last menstrual period. Pregnancy was defined as normal when no antenatal complications occurred and fetal birth weight varied between the 10th-90th percentile according to the tables of Kloosterman (14) corrections being made for maternal parity and fetal sex. Consecutive measurement of the fetal B P D and chest area was performed once in each patient of the normal group and once or more in patients of the complicated group.

RESULTS

pregnancy

In order to be able to compare fetal head area measurements with fetal chest area measurements the B P D² instead of the B P D was taken into account.

Fetal head and chest area measurements were plotted against gestational age (Figs 2 and 3). The mean head area varied from 45.0 cm² at 25 weeks to 98.0 cm² at 40 weeks gestation. After 37 weeks there appears to be a decrease in the growth rate of the head area. The mean chest area varied from 31.3 cm² at 25 weeks to 94.3 cm² at 41 weeks. Mean values and standard deviations of head and chest area are given in Table II. In Fig. 4 fetal head area values are plotted against fetal chest area values in order to evaluate normal head-to-chest relation between 25 and 41 weeks gestation.

Complicated pregnancy

Fetal head and chest area values are evaluated against fetal birth weight. This was classified ac-

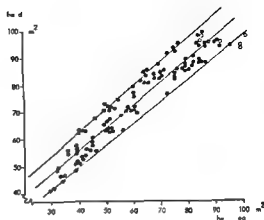
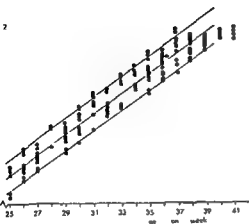
cording to the tables of Kloosterman (14) corrections being made for maternal parity and fetal sex. Fetal B P D measurements which were performed before 25 weeks of gestation were initially plotted against Campbell's B P D growth curve (3). When more than one ultrasonic measurement of the fetal B P D and chest area was performed only the most recent measurement was taken into account. The difference between this measurement and actual delivery varied from one day to 6 weeks with a mean duration of 24 days.

In 18 cases fetal birth weight was situated between the 10th-90th percentile (normal) in 79 cases below the 10th percentile (small-for-dates) and in 12 cases above the 90th percentile (large-for-dates) weight of gestation. Details are given in Table III.

In the normal weight group both fetal head and chest area were within the normal range in the majority of the cases. In 3 cases however head area was within and chest area just below the normal range. In the small-for-dates group both fetal head and chest area were within the normal range in one case. In the remaining 28 cases 2 types of growth retardation could be distinguished. Type 1 (n=7) was characterized by a small fetal head and chest area with a normal head-to-chest ratio.

Table II Mean fetal head area and chest area values (\bar{x}) with standard deviations (S D) between 25 and 41 weeks of menstrual age in normal pregnancy

Gestational age (wks)	No of cases	Mean head area (=B P D ²) in cm ²		Mean chest area (cm ²)	
		\bar{x}	S D	\bar{x}	S D
25	8	45.0	3.9	31.3	2.1
26	10	51.6	3.7	37.7	2.4
27	10	57.2	4.3	37.8	2.4
28	6	60.8	3.4	44.3	2.3
29	10	61.6	3.4	45.3	2.3
30	10	65.9	3.8	52.0	2.4
31	10	71.1	4.3	51.9	2.4
32	9	73.6	4.2	57.4	2.4
33	7	77.4	5.5	58.0	2.4
34	7	82.1	5.3	68.4	2.4
35	12	83.3	6.3	77.6	2.4
36	13	86.0	3.3	80.5	2.4
37	10	97.8	5.1	81.7	2.4
38	11	97.6	3.7	87.4	2.4
39	8	94.2	7.4	90.3	2.4
40	5	98.0	7.3	94.3	2.4
41	6	96.0	5.6		



Figs 2 3 and 4 The 10th 50th and 90th percentile of the normal curve for fetal head area (Fig 2) fetal chest area (Fig 3) and fetal head to chest relationship (Fig 4) between 25 and 39 weeks gestation. The open circles in Fig 4 represent the head and chest area values at 40 and 41 weeks of gestation

indicating a symmetric growth retardation. This type of growth retardation usually started between the 22nd and 28th week of gestation.

The second type ($n=21$) showed a normal to small fetal head area and small chest area with an abnormal head to chest relationship indicating an asymmetrical growth retardation. This type of growth retardation started during the 3rd trimester in all cases. Figs 5 and 6 show a longitudinal follow up of fetal head and chest area in a patient from the first and second sub group respectively.

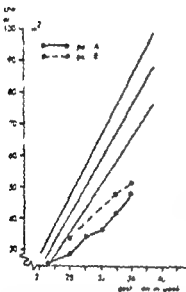
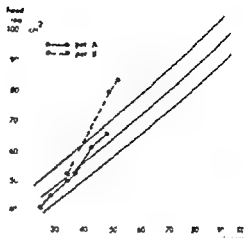
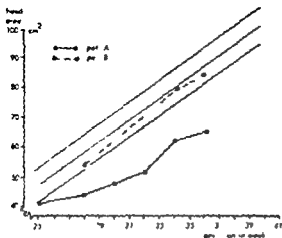
Fig 7 shows the head to chest relationship in both patients.

In the large for-dates group a normal fetal head area was related to a normal chest area in two cases, the remaining 10 cases head size was normal or large ($n=3$) in combination with a large chest area. Head-to-chest relationship was always abnormal due to a relatively large chest.

DISCUSSION

From our study on normal pregnancies it can be demonstrated that in the second trimester the fetal head area is larger than the fetal chest area. In the third trimester because of accumulation of subcutaneous fat and soft tissue during this part of pregnancy the chest area catches up with the head. This can also be seen in the head to chest relationship curve. At term both dimensions are more or less of the same magnitude. The increase in head area seems to tail off beyond 37–38 weeks. In our study of small for-dates 2 types of fetal growth retardation could be recognized (Table III). Figs 5 and 7. Similar patterns were described by Campbell (4) and Hansmann (11).

Asymmetrical growth retardation has been observed in experimental studies on impairment of vascular blood supply to the foetus in rats (19), sheep (7) and in monkeys (12, 13, 16). In these



Figs 5, 6 and 7 Longitudinal follow-up of fetal head area (Fig 5) fetal head to-chest relationship (Fig 6) and fetal head to-chest relationship (Fig 7) in a case of symmetrical (Pat. A) and a case of asymmetrical (Pat. B) fetal growth retardation

idies brain to body ratio was elevated suggesting brain sparing effect. This might be due to a preferential redistribution of well-oxygenated blood to the fetal cerebrum. Gruenwald's (9) pathologic findings in the human foetus confirm this type of growth retardation in relation to utero-placental vascular insufficiency.

Symmetrical growth retardation is considered the outcome of a reduced growth potential as observed in malnourished rats (15-21) and malnourished guinea pigs (6-18). In the human foetus this type of growth retardation is seen after viral infection in early pregnancy and in the presence of chromosomal aberrations. There is a permanent loss of cells and the fetal brain is not spared. In our study 10 out of the 21 cases of asymmetrical growth retardation were associated with pre-eclamptic toxæmia or essential hypertension. In the remaining 11 cases no vascular complications were observed. In our group of symmetrical growth retardation twice

a vascular factor could be demonstrated once it was mitral stenosis and once a bicornuate uterus. In the remaining 3 cases no apparent reason for the slow-down in fetal growth was found. It seems likely that fetal malnutrition as a result of a reduced maternal caloric uptake plays an important role in our part of the world. According to our data, the asymmetrical type of growth retardation always started in the last trimester of pregnancy in contrast to the symmetrical type which started as early as 10 weeks of gestation. The time of pregnancy in which fetal growth retardation starts may be of importance in relation to the long term outcome of the foetus. Dobbing and Sands (8) described two phases of human brain growth. The first phase from 10 to 20 weeks of gestation consists of neuroblast proliferation; the second phase from 25 weeks of gestation up to the second year of life reflects glial proliferation.

A long term follow-up of the physical and mental

Table III Head area chest area head to-chest relationship and antenatal complications in the various weight categories in 59 complicated pregnancies

of 5	Head (B P D) ^a area (cm ²)	Chest area (cm ²)	Head to-chest relationship	Pregnancy duration (wks) during last ultrasonic measurement	Antenatal complications
Normal weight group					
Normal	Normal	Normal	Normal	33-39	P E T 9x Essential hypertension 4x Recurrent vaginal bleeding 2x
Normal	<10th percentile	<10th percentile	Abnormal	34-36	P E T
D group ^b					
Normal	Normal	Normal	Normal	37	P E T
<10th percentile	<10th percentile	<10th percentile	Normal	32-38	P E T 1x Essential hypertension 1x Mitral stenosis 1x Bicornuate uterus 1x None 3x
Normal or <10th percentile	<10th percentile	<10th percentile	Abnormal	33-39	P E T 9x Essential hypertension 1x None 11x
L group					
Normal	Normal	Normal	Normal	35-36	None
Normal	>90th percentile	>90th percentile	Abnormal	37-40	Recurrent vaginal bleeding 1x Fetal hydrops 1x Abnormal G T T 2x None 3x
>90th percentile	>90th percentile	>90th percentile	Abnormal	33-38	Abnormal G T T

^a T = pre-eclamptic toxæmia S F D = small-for-dates L F H = large for-dates G T T = glucose tolerance test.

development of the small-for-dates newborn will be needed to evaluate the pattern of growth retardation described in this paper. In the 10 cases of growth acceleration head to-chest relationship was asymmetrical i.e. the fetal chest was relatively larger than the fetal head. This finding suggests that fetal fatty tissue is particularly affected in growth acceleration. In 5 out of these 10 cases an abnormal glucose tolerance test was found. It can be concluded that consecutive ultrasonic measurement of fetal head and chest size provides valuable information on the pattern of a developing fetal growth retardation or acceleration. Further consecutive measurement of fetal head and chest size will improve the pick up rate of asymmetrical growth retarded or accelerated infants when compared with the estimation of fetal head size alone.

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ENDOMETRIAL HISTOLOGY AND CIRCULATING LEVELS OF MEDROXYPROGESTERONE ACETATE (MPA) ESTRADIOL FSH AND LH IN WOMEN WITH MPA INDUCED AMENORRHOEA COMPARED WITH WOMEN WITH SECONDARY AMENORRHOEA

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act Circulating levels of medroxyprogesterone (MPA) estradiol progesterone and gonadotropins were determined in 11 women on long term treatment depot MPA (Depo-Provera® DMPA) 150 mg i m / 12th week as a contraceptive. The women had amenorrhoea due to the treatment. Endometrial biopsy performed one week after injection and at the end of the 1st week period. Blood samples were taken on the occasions. The findings were compared with those of untreated women having secondary amenorrhoea. MPA was still detectable in serum and the end of the 1st week period. Endometrial biopsies showed gestagenic effect in the second as well as in the first biopsy. No MPA detectable in the untreated women with amenorrhoea. No gestagenic effects could be demonstrated in their cases. The estradiol levels in the DMPA group were in the range of the early follicular phase of a normal menstrual cycle and showed a significant rise at the end of the 1st week period. On the last sampling occasion the estradiol levels did not differ from those in the untreated women with secondary amenorrhoea. The levels of progesterone and gonadotropins were in the range of the early follicular phase in both groups. These observations support that DMPA 150 mg i m every 12th week is a depot preparation with prolonged effect and inhibits ovulation. It produces endometrial changes by means of biological active serum concentrations throughout the 1st week period.

pure gestagenic compound medroxyprogesterone acetate in a depot preparation (DMPA) has been in use as a contraceptive agent for about ten years. It is generally administered in a dose of 150 mg intramuscularly every 12th week. The large number of reports on its efficacy, acceptability and effects have recently been reviewed (16, 18).

DMPA 150 mg i m every 12th week is a contraceptive method as reliable as the use of combined oral contraceptive pills and has only few side-effects, mainly bleeding disturbances. During treatment there is during the first months unpredictable uterine bleeding which gradually ceases and most of the women thereafter develop amenorrhoea.

Despite the extensive use of DMPA as a contraceptive, the mode of action of the drug is not fully understood. Its main mode of action seems to be inhibition of ovulation as it is shown (12, 13) that it for at least three months abolishes the mid-cycle surge of the luteinizing hormone (LH). In addition it has a gestagenic effect on the cervical mucus and the endometrium.

The period of time during which ovulation is inhibited is sometimes prolonged. In a previous paper (5) we raised the question whether this prolonged inhibition of ovulation is due to individual differences in the rate of absorption at the site of injection, i.e. due to an actual drug effect demonstrable for more than 12 weeks, or due to hypothalamic suppression of varying duration caused by high initial serum levels of the drug which soon decrease to non-detectable concentrations. The inhibition of ovulation within the scheduled 12 week period might also be due rather to such a prolonged hypothalamic suppression than to circulating levels of MPA at the end of the period.

The aim of the present study is to clarify whether there are detectable levels of MPA in serum or demonstrable effects on the endometrium at the end of



Fig 1 Endometrial biopsy taken one week after injection in a DMPA treated patient showing atrophic endometrium with few and small glands having low epithelium and some stromal oedema (H E $\times 70$)

12 week period in women with amenorrhoea using DMPA 150 mg i m every 12th week as a contraceptive and to compare these findings with untreated women having secondary amenorrhoea.

MATERIAL AND METHODS

A total of 23 women were investigated. Eleven of the women aged 24-43 (mean 31.8) received injections of DMPA (Depo-Provera[®]) every 12th week as a contraceptive. They were studied during the 12 week period after having received 8 to 11 injections of DMPA (mean 10.8 injections) without interruption of the treatment and they all had amenorrhoea for at least 10 months. During the period of the highest serum concentrations of MPA (16) blood samples from an antecubital vein were obtained on day 6-15 (mean 8.3) after the injection of DMPA. On the same day endometrial biopsy specimens were obtained. At the end of the 12 week period immediately before the subsequent injection of DMPA the blood sampling and endometrial biopsy were repeated.

The other 12 women aged 19-39 (mean 25.7) were all patients with secondary amenorrhoea from different

causes. The amenorrhoea had lasted more than 12 months. These women had never received any injectable and did not receive any therapy for their amenorrhoea during the period of investigation. In these women a blood sample was taken and on the same day an endometrial biopsy was performed.

All the blood samples were treated in the same way. The blood was collected partly in empty centrifuge tubes and partly in centrifuge tubes containing EDTA. The samples were centrifuged and the serum and plasma respectively were removed and stored at -20°C until analysed.

The plasma samples were used for the assay of FSH and the serum samples were used for the assay of MPA, estradiol and progesterone.

The endometrial biopsy specimens were obtained without anaesthesia or dilatation of the cervix by curette according to Genell. Biopsies were taken from the anterior and posterior wall of the uterine cavity. The specimens were immediately fixed in 10% formalin, after preparation stained with haematoxylin and eosin. The sections were examined histologically according to the morphological criteria of Noyes et al (15) although modified. The mitotic activity, the vacuolation of the glandular epithelium and the secretion were classified as absent or present. The use of these parameters as used by Noyes et al (19) and others (7) was omitted.

Medroxyprogesterone acetate was assayed by radioimmunoassay (6). In 1-1.0 ml of serum was extracted with 1 ml of diethyl ether. No corrections were made for procedural losses. The antiserum against MPA was from Dr Karim Upjohn Co, Kalamazoo, USA.

Estradiol in serum was assayed by radioimmunoassay according to Edqvist & Johansson (11).

Progesterone was determined by radioimmunoassay according to Thorneycroft & Stone (70).

Plasma LH and FSH were determined in double antibody radioimmunoassays. The previously reported in detail (19). In this assay standard for LH was equivalent to 55 ng of LER 10 mIU of the second IRP hMG and 1 ng of the standard for FSH was equivalent to 98 ng of LER 907 or 13 ng of the second IRP hMG. The average coefficients of the means of triplicated determination were 5.7 per cent for LH and FSH respectively. The range 1.0-20.0 ng/ml. The normal range of LH and FSH levels during the follicular phase in healthy women was 0.5 to 3.0 ng/ml.

RESULTS

MPA The serum concentration of MPA in the DMPA treated group about one week after injection was 3.57 ± 0.51 (mean \pm S.E.) ng/ml and decreased to 0.6 ± 0.1 ng/ml at the end of the 12 week period. The decrease was highly significant ($P < 0.001$). In the patients with secondary amenorrhoea without having received MPA no concentration of MPA were detectable in serum.

Estradiol The concentration of estradiol in

Histological appearance of endometrial biopsy specimens in the two groups of women

		Patients given DMPA		Patients with secondary amenorrhoea
		1st biopsy	2nd biopsy	
no. of biopsies successful		11	11	12
activity of glandular epithelium	Absent	10	9	4
	Present	0	2	5
glandular pseudostratification	Absent	10	9	1
	Present	0	2	8
glandular vacuolation	Absent	8	6	7
	Present	2	5	2
glandular secretion	Absent	8	5	7
	Present	2	6	2
oedema	Absent	4	5	8
	+	6	5	1
	++	0	1	0
decidual reaction	Absent	1	1	8
	+	7	4	1
	++	2	6	0

A group about one week after injection was 1.9 pg/ml and rose significantly ($p < 0.025$) to $55.4 \pm 10.6 \text{ pg/ml}$ at the end of the investigation. In the untreated group of patients with secondary amenorrhoea the estradiol concentration was 4.5 pg/ml and this level did not significantly ($p > 0.05$) differ from the estradiol level in the treated women at the end of the 12 week period. The levels are in the range of the early follicular phase of ovulatory women.

Progesterone Serum levels of progesterone were 0.6 ng/ml in the DMPA group one week after injection as well as at the end of the investigation serum levels of progesterone were below 0.6 ng/ml also in the patients with secondary amenorrhoea.

Gonadotropins The levels of gonadotropins on occasions in the DMPA group as well as in the untreated women with amenorrhoea were in the range of the follicular phase in normally menstruating women. No difference between the concentrations on the first ($\text{LH } 0.9 \pm 0.1 \text{ ng/ml}$ and $2.5 \pm 0.2 \text{ ng/ml}$) and the second ($\text{LH } 0.95 \pm 0.1 \text{ ng/ml}$ and $\text{FSH } 2.2 \pm 0.2 \text{ ng/ml}$) sampling occasion in the DMPA treated group was found. The gonadotropin levels in the untreated group of patients with secondary amenorrhoea were for LH $0.9 \pm 0.1 \text{ ng/ml}$ and for FSH $2.1 \pm 0.2 \text{ ng/ml}$ and these levels

did not differ from those seen in the women receiving DMPA injections.

Endometrial biopsy specimens The biopsies from patients given DMPA showed a marked atrophy of the endometrium (Fig. 1). The glands were few with narrow lumina and the epithelium flattened and single layered with a marked reduction of the mitotic activity. The stroma showed varying degree of oedema and decidualization. The stromal changes however were less pronounced and tended to be rather focal. When present decidual reaction was usually seen around the blood vessels. In general the first and the second biopsy showed only minor histological differences.

Table 1 summarizes the histological findings. As can be seen the stromal decidual change tended to be somewhat more marked at the end of the 12 week period. While invariably absent on the first biopsy occasion mitotic figures and pseudostratification of the epithelial cells appeared in 2 cases at the end of the treatment period. Signs of a weak secretory activity were noticed in 2 out of 10 cases on the first biopsy occasion but at the end of the investigation epithelial vacuolation had appeared in 5 and glandular secretion in 6 out of 11 biopsies.

The histological diagnosis made on biopsies from the amenorrhoeic patients were as follows: resting endometrium or weak proliferative activity in 5



Fig. 2 Endometrial biopsy from an amenorrhoeic patient showing late proliferative phase (H.E. $\times 20$)

cases normal proliferative phase in 2 cases and hyperplastic endometrium in 2 cases.

Eosinophilic secretion like material was observed in some glands in biopsies from two patients and basal vacuolation was seen in occasional epithelial cells in biopsies from another two cases belonging to the series with proliferative activity or resting endometrium. These changes were very weak and considered inconclusive for diagnosing secretory activity (Table I).

In 3 cases the biopsy specimens were inadequate for diagnosis. The biopsies invariably differed histologically from those taken from the DMPA treated women (Table I and Fig. 2).

DISCUSSION

The present investigation showed detectable concentrations of MPA in serum at the end of the 12 week period in women receiving 150 mg DMPA every 12th week as a contraceptive agent. Furthermore endometrial biopsies showed mainly gesta-

genic effects in specimens obtained at the end of the 12 week period as well as in specimens taken one week after the injection of DMPA.

These observations support that DMPA is a depot preparation with prolonged effect after drug injected and that this prolonged effect lasts for at least 12 weeks after injection of 150 mg DMPA i.m. No detectable concentrations of MPA were found in the untreated women with amenorrhoea and no gestagenic effects were found in endometrial biopsy specimens.

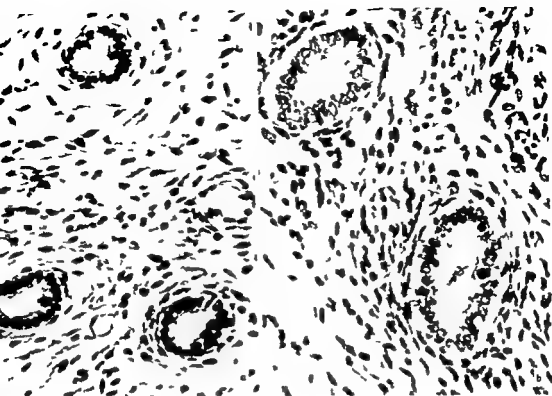
It is recently shown (Victor & Johansson, unpublished) that even low concentrations of 0.5 and 1.0 ng/ml of MPA in serum are able to inhibit ovulation. Therefore it seems probable that the main mode of action of DMPA in the mentioned dose is exerted by means of pharmacologically acting serum concentrations of MPA throughout the 12 week period.

Gestagenic features can be shown at the end of the period clearly different from the endometrial features in the patients with secondary amenorrhoea. This strongly supports that the endometrial changes in patients receiving DMPA as a contraceptive result mainly from a direct effect of continued progestational stimulation and to a lesser extent from the low production of endogenous estrogens which did not differ significantly between the two groups.

The endometrial histology in the DMPA treated women was mainly in accordance with previous studies on endometrial effects of Depo-Provera (2, 10, 14).

In some cases signs of secretory activity appeared in the endometrial biopsy taken at the end of the 12 week period (Fig. 3a and b). Previous studies (3, 11) have shown the importance of progesterone for the appearance of progesterone receptors in the endometrium. In the present investigation a significant rise in the serum concentration of estradiol at the end of the 12 week period was found. This increase might be sufficient to activate progesterone receptors to produce histological secretory changes in the presence of biologically active serum levels of MPA. The estradiol levels in the patient whose biopsies are shown in Fig. 3 were 13 and 46 pg/ml respectively on the two occasions.

The finding of biologically active concentrations of MPA in serum at the end of the 12 week period does not exclude the possibility that hypothyroidism suppression induced by initially high con-



Endometrial biopsy from a patient treated with MPA. No signs of secretion in the first biopsy (a) basal vacuolation in the second (b) (H.E. $\times 448$)

of MPA in serum can play a role in the profound inhibition of ovulation. Kirton & Cornette measured serum levels of MPA in three patients 60 days after one single injection of 150 mg MPA. The first ovulation after injection of MPA occurred at very different levels of MPA in serum. In one subject ovulation did not occur for more than 100 days after drug concentration was non-detectable. Thus, other mechanisms than actual effect is likely to be involved.

The depressed levels of estradiol in serum about 1 week after injection of DMPA corresponding concentrations in the lower part of the normal range in the normal early follicular phase (9) and the subsequent rise at the end of the 12 week period are in accordance with previously published investigations (5). In agreement with previous reports (4) the basal secretion of gonadotropins was not affected during treatment with DMPA and the plasma levels did not differ from those in untreated women with secondary amenorrhoea.

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A RADIOIMMUNOLOGICAL METHOD FOR OESTRONE

Plasma Levels of Non conjugated Oestrone during Uncomplicated Pregnancy

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Abstract A specific antiserum to oestrone¹ has been used to develop a radioimmunoassay. No chromatographic separation step was included. A small amount of plasma (0.15 ml) was extracted once with diethyl ether. The method was used to estimate plasma levels of non-conjugated oestrone in women during uncomplicated late pregnancy. The oestrone plasma concentrations showed a gradual increase from a mean of 4 ng/ml in the 27th week to 1 ng/ml in the 41st week. Great individual variations were found. No systematic changes of unconjugated oestrone plasma concentrations were noticed during a 24-hour period in late pregnancy. Short term studies revealed large and rapid fluctuations may appear in the levels of unconjugated oestrone during pregnancy.

Many studies have been performed on biosynthesis, urinary excretion and plasma concentrations of oestrogen hormones during human pregnancy. The relation between mother, fetus and placenta in oestrogen synthesis and metabolism has been elucidated. The introduction of the concept of the foetal unit (7) has turned out to be fruitful for understanding of the oestrogen metabolism in human pregnancy. Still the knowledge of the biological role of oestrogens in gestation is limited.

The following abbreviations and trivial names are used: oestrone=3-hydroxy-1,3,5(10)-oestratrien-17-one; oestradiol-17 β =1,3,5(10)-oestratriene-3,17 β -diol; oestrinol=1,3,5(10)-oestratriene-3,16 α ,17 β -triol; androstenedione=androstene-3,17-dione; cortisol=11 β ,17 α ,21-trihydroxy-4-pregnene-3-one; 17 α -hydroxyprogesterone=17 α -hydroxy-4-pregnene-3,20-dione; progesterone=4-pregnene-3,20-dione; testosterone=17 β -hydroxy-4-androstene-3-one; 16 α -hydroxyoestrone=3,16 α -dihydroxy-1,3,5-oestratrien-17-one.

Oestrone plasma or blood levels have earlier been studied by various methods. Roy & Brown (26) used a colorimetric method. Preedy & Aitken (25) and Itrich (16) described fluorometric methods. Svendsen (30) and Baird (4) developed double isotopic derivative methods. Later the technique of gas chromatography was used by Adlercreutz (1) and Fischer Rasmussen (12). Attal et al (3) described a way for determination of oestrone with electron capture detection. In the nineteen seventies a competitive protein binding technique was used for the estimation of oestrone plasma levels (14, 28, 33).

In recent years radio-immunoassays have been developed for measurements of oestrogens in blood. Since no specific antibody to oestrone has been available, previous methods have included a separation step. In most cases some kind of chromatography (10, 11, 18, 19, 22, 24, 34, 35).

In 1974 Lindberg et al (22) published radio-immunological methods for estimating unconjugated oestrone, oestradiol-17 β and oestrinol. Their report included studies of oestrogen plasma levels in women with uncomplicated and high risk pregnancies (20, 21). At that time no specific oestrone antibody was available. The present investigation was done with the same specific antiserum as that used by Brenner et al (5) to develop a method for the estimation of oestrone in plasma during pregnancy. Thus it will be a complement to the works of Lindberg et al. This report will present the method and give plasma levels of non-conjugated oestrone during late normal pregnancy. Circadian and short term variations were also studied.

Table 1 Per cent crossreaction with the oestrone antibody

Test substance	Per cent crossreaction
Oestrone	100
Androstenedione	<0.1
Cortisol	<0.1
17 α hydroxyprogesterone	<0.1
Oestradiol 17 β	3.3
Oestrol	1.4
Progesterone	<0.1
Testosterone	<0.1

MATERIAL AND METHODS

Steroids

(4,6,7)- ^3H oestrone (S.A. 115 Ci/mmol) was obtained from New England Nuclear Corp. Boston USA. Non-radioactive oestrone, oestradiol 17 β , oestrol, 17 α hydroxyprogesterone, androstenedione, progesterone and testosterone were supplied by Ikapharm, Tamal-Gan, Israel. Non-radioactive cortisol was obtained from Sigma Chemical Company, St. Louis, Mo. USA. The antiserum was supplied by Dr. Jean Pierre Raynaud, the Roussel Uclaf Research Centre, France. Without any pretreatment the antibody was diluted to 1:10,000 in phosphate buffered saline (PBS) containing 0.1% gelatin and stored at 4°C.

Reagents

Special chemicals were obtained from the following sources: Charcoal (Carbo medicinalis Nord) Apotekarnas AB ADA, Gothenburg, Sweden; Dextran T70 from Pharmacia Fine Chemicals, Uppsala, Sweden; (Favonigelatin) from AB Törsleff Co., Stockholm, Sweden; Naphthalene from Packard Inc., Downer Grove, USA; Omnifluor from New England Nuclear Corp., Boston, USA.

Reagent grade absolute ethanol, dioxane and diethyl ether from freshly opened cans were used without any additional purification. Phosphate buffered saline containing 0.01 M sodium phosphate (pH 7), 0.14 M sodium chloride, 0.01% merthiolate was used as the diluent solution. Buffer containing 0.1% gelatin was used to dilute the antibodies. Dextran-coated charcoal suspension was made by adding 2.5 g charcoal and 0.25 g Dextran T70 to 1000 ml PBS.

Assay method

Hotchkiss *et al.* (15) and Edqvist & Johansson (10) described a method for measurement of oestradiol 17 β . With minor modifications the same procedure was used for the oestrone measurement in the present investigation. When analysing plasma from pregnant women volumes of 0.01–0.15 ml were used. Plasma was extracted with 1 ml of diethyl ether by shaking on a Vortex mixer. The ether solution was decanted after freezing of the aqueous layer. The recovery after this extraction step was 75–80%. When

the method was used for plasma from women a volume of 0.4 ml was used. Plasma was extracted with 10 volumes of diethyl ether by intensive shaking for 1 min. The supernatant was withdrawn by syringe. The recovery with this procedure was 90–95%. The technique to withdraw the diethyl ether gave higher recovery also when smaller plasma volumes were used. This method was then not acceptable.

The supernatant was evaporated to dryness. 1 ml of the antibody solution was added to each tube. The tubes were briefly shaken and incubated for 30 min at room temperature. After incubation 100 μ l of the solution was added to each tube. This solution contained 400 pg (4,6,7)- ^3H oestrone (~ 1.5 μCi). The contents of the tubes were briefly mixed and incubated at 4°C in a refrigerator. Free and bound steroids were separated by the dextran-coated charcoal technique.

Counting was performed on Packard Tri-Carb scintillation spectrometers (Model 3310 and 3330). Counting efficiency was 25 and 40% respectively. The scintillation solution contained 100 mg naphthalene in 1000 ml dioxane.

Standard samples made up with 25, 50, 100, 250, 500 and 1000 pg in duplicate, were counted simultaneously for each set of samples.

Evaluation of the method

Specificity The specificity of the antibody was assayed by known amounts of different test steroids. The percentage cross reaction at 50% displacement of the tracer. The results are given in Table 1.

Different volumes of pooled plasma samples from menopausal and postpartum (5–6 days after delivery) women were extracted with 10 volumes of diethyl ether and analysed. The values obtained were superimposed.

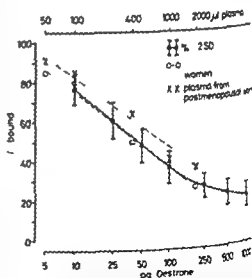


Fig. 1 The mean and spread (\pm S.D.) of 57 curves prepared over a period of 16 months. 0 (no added unlabelled steroid) has been set to 100.

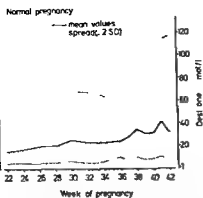


Fig. 1. Plasma levels of non-conjugated oestrone during complicated pregnancy. The spread (± 2 S.D.) was calculated after a logarithmic transformation of the original values. In this and the following figures a scale of nmol/l is used.

the standard curve of oestrone (Fig. 1). The oestrone measured in plasma from postpartum women was 71 μ l and the level in plasma from postmenopausal women was 45 pg/ml.

Accuracy. The accuracy of the method appears from Table II where recovery experiments are shown. Known amounts of oestrone were added to 50 μ l of plasma from postpartum women. Acceptable accuracy was obtained in the working range.

Sensitivity. It was found that values of 1 pg of oestrone were significantly different from zero (Table II). Samples assayed were well above the limit of detection. The effect of reagent blank was minimized by adding of diethyl ether to the standard tubes. The plasma level was considered as negligible (Fig. 1).

Precision. The within assay variation was calculated as the differences between duplicate determinations of plasma samples according to Snedecor (29). The results are given in Table III.

For calculation of the between assay variation a plasma containing a known amount of oestrone (10 ng/ml) was assayed. 57 duplicate measurements were performed. The coefficient of variation was 11.1%. The variation between standard curves over a long period (Fig. 1) indicated the importance of preparing standard curves with each set of samples assayed.

Results. Twenty women were followed from the 22nd week of pregnancy until delivery. Sampling was performed every second week from the 22nd to the 36th week and then every week. Samples were also collected from a number of women attending the municipal antenatal clinic in Uppsala in the 1st, 24th, 26th, 36th, 37th, 38th etc. week of pregnancy. Most women contributed only one sample but a few two to three at different stages of gestation. All women were in general good health with regular menstrual cycles. The date for the last menstrual period was known. All pregnancies were uncomplicated and resulted in the

Table II. Recovery of oestrone added to 50 μ l of plasma from postpartum women.

Correction was made for procedural losses. CV is coefficient of variation. All assays were performed at each level.

Amount of oestrone added in pg	Per cent recovery	
	Mean with 95% confidence limits	CV
1	236 \pm 151	61
2.5	113 \pm 41	34.8
5	106 \pm 25	22.9
10	108 \pm 48	42.5
25	112 \pm 9	7.7
50	95 \pm 11	11.3
100	77 \pm 5	6.3
250	71 \pm 23	30.3
500	84 \pm 22	24.6

birth of single healthy infants with a birth weight exceeding 2500 g. No significant differences in the plasma oestrone levels were found between these two series of women. The results were thus pooled for calculating means and normal limits in the various weeks of pregnancy.

Circadian variations in oestrone plasma levels were studied in six women. In four women venous samples were taken every fourth hour in two cases every second hour. Two patients had mild pre-eclampsia. At the time of sampling, however, the symptoms had disappeared.

Short term variations were studied in five women. Samples were collected every five minutes during one hour. Four of these women had mild pre-eclampsia.

Venous blood was collected in heparinized tubes. Plasma was separated by centrifugation and stored at -20°C until assayed.

Statistical methods

As the distribution of the values in the various weeks of pregnancy displayed a positive skewness, normal limits were calculated after a logarithmic transformation of the original values $Y = \ln x$, where x is the observed value. Upper limit = $e^{Y + 2s}$ and lower limit = $e^{Y - 2s}$ (13).

Table III. The precision of the oestrone measurements at different levels.

Range (ng/ml)	Coefficient of variation (%)	Number of duplicate determinations
0-5	17.8	15
5-10	12.9	46
10-15	11.6	72
15-20	14.7	43
>20	16.0	53

Table IV Plasma levels in ng/ml of non-conjugated oestrone during uncomplicated pregnancy

Upper and lower limits (± 2 S.D.) were calculated after a logarithmic transformation of the original values

Week of pregnancy	No. of samples	Mean	Upper limit	Lower limit
22	30	4.1	10.2	1.3
24	30	4.7	12.2	1.3
26	29	5.4	16.8	1.2
28	30	5.4	14.7	1.4
30	30	6.8	18.6	1.8
32	30	6.3	18.4	1.4
34	30	6.2	17.4	1.6
36	29	6.6	13.3	2.8
37	30	7.7	20.6	2.0
38	30	9.4	24.2	2.8
39	29	8.4	22.8	2.2
40	29	8.6	22.8	2.4
41	30	11.1	30.7	2.9
42	15	8.5	31.5	1.4

RESULTS

The plasma levels of non-conjugated oestrone during uncomplicated pregnancy are shown in Table IV. Fig. 2. From week 22 to week 36 there was a slow increase from a mean of 4.1 ng/ml to 6.6 ng/ml. Thereafter the mean level increased somewhat more rapidly to 11.1 ng/ml in the 41st week. In week 42 the mean level decreased to 8.5 ng/ml. At this stage of gestation, however, only 15 samples were available for analysis. The lower normal limit showed a slow increase from 1.3 ng/ml in the 22nd week to 2.9 ng/ml in the 41st week. The upper limit increased about threefold from about 10 ng/ml to 30 ng/ml during the same period.

Plasma concentrations of non-conjugated oes-

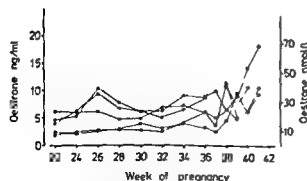


Fig. 3 Plasma levels of unconjugated oestrone in five healthy women followed throughout pregnancy. The remaining ten patients showed similar patterns.

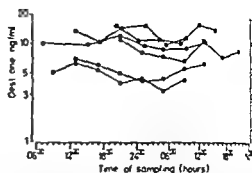


Fig. 4 Variations in the plasma levels of non-conjugated oestrone during 20-30 hour periods in six pregnant women in the last trimester. A logarithmic scale is used.

trone recorded for individual women throughout pregnancy are graphically described in Fig. 3. Generally, the values of the same women were to be found in the same range of the normal curve throughout pregnancy.

Circadian variations in plasma levels of non-conjugated oestrone during 20-30 hour periods are shown in Fig. 4. No evident changes corresponding to rest or meals could be found. The maximal difference between oestrone levels measured in the same individual was 4.8 ng/ml. The results of short-term studies are given in Fig. 5. The maximal difference between concentrations measured in the same individual during one hour was 11.6 ng/ml. In one woman the oestrone values were found to be below the lower limit of the normal curve. This patient had mild pre-eclampsia but showed no symptoms at the time of sampling. Later, she gave birth to a healthy infant weighing 3020 g.

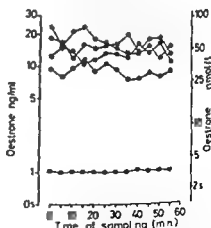


Fig. 5 Variations of non-conjugated oestrone during one hour in five pregnant women in the last trimester. A logarithmic scale is used.

DISCUSSION

In previously published radio-immunoassays for oestrone have included a laborious column chromatography (5 10 11 24 34 35). In efforts to combine rapid techniques some authors omitted the chromatography (10 11) and measured immunoreactive oestrogens rather than oestrone with fairly specific antisera. In the present study a radioimmunological method for the assay of plasma oestrone omitting the chromatographic step has been developed. Due to the high specificity of oestrone antiserum used in the present study oestrone plasma concentrations can be measured without prior separation of the plasma oestrogens as was true also with a method recently published by Doerr (8). The oestrone antiserum used in our preliminary crossreacts with oestradiol 17β and oestrinol to a minor degree. Since the plasma levels of unconjugated oestradiol 17β and oestrinol are substantial during late pregnancy (20) the method may give a slight over estimation of the plasma oestrone levels. The results are however present without correction for this over estimation and without correction for procedural losses. If corrections were done only small differences in the final results should be noticed. By the omission of these corrections the procedure becomes faster and simpler. In another publication (5) a crossreactivity of 3 per cent with 16α hydroxyoestrone was reported for this antiserum. Since the plasma level of unconjugated 16α hydroxyoestrone is low during pregnancy (2) the interference of this hormone is negligible.

The plasma levels of oestrogens during pregnancy have been the subject of many investigations. Most of these reports however have dealt with oestrinol and oestradiol. For references see (20). Most investigations of the blood concentrations of oestrone during pregnancy have revealed a slow increase of the oestrone level throughout pregnancy. This was true for unconjugated (6 9 20 23) as well as total (unconjugated+conjugated) oestrone (17 27). Svendsen & Sorensen (31) found an increase in oestrone levels up to the last stage of pregnancy where the concentrations remained constant or showed a weak tendency to decrease. Rasmussen (12) presented an extensive investigation of total oestrone in plasma. He found no significant increase in plasma oestrone during late pregnancy. Three women however showed very

high values. If these values were neglected a significant increase was evident.

Our results are in agreement with previous studies in that we found a slow increase during late pregnancy and large individual variations. Our estimations of non conjugated oestrone in plasma are in the same range as the results of Svendsen & Sorensen (31), Tulchinsky et al (32), Loraux et al (23), Lindberg et al (20), de Hertogh et al (6) and Dumont et al (9). These groups used a double isotopic derivative method (31), a radioligand assay (32) and radio immunological assays (6 9 20 23) respectively. Roy & Mackay (27) could not demonstrate any significant changes of total oestrone levels in blood during a 24 hour period. Our circadian and shortterm studies reveal that the levels of unconjugated oestrone may show large and rapid variations. No systematic patterns in the fluctuations could be noticed during a 24 hour period. The rapid variations may be explained by the assumption that the degree of conjugation undergoes rapid changes.

The aim of this study was to develop a simple and reliable method for the measurement of unconjugated oestrone. It is known that the main part of plasma oestrone in human pregnancy is conjugated (23). Maybe the plasma levels of unconjugated and conjugated oestrone would give a more representative picture of the oestrone production in placenta. By introducing a hydrolysis however our procedure would become more time-consuming and thus less suited for clinical purposes.

This description of plasma levels of oestrone during normal pregnancy will serve as background data for investigations of oestrone in pathological pregnancies.

ACKNOWLEDGEMENTS

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HETEROLOGOUS GROWTH OF HUMAN OVARIAN CANCER

A New in vivo Testing System

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Abstract Successful heterotransplantation of ovarian cancer in the mouse mutant *nude* is described with serial subinoculation in one case to date. Light and electron microscopic examination of this tumour, a poorly differentiated adenocarcinoma, has not revealed any alterations in its morphology after six passages. Tumour-bearing mice have been treated with ThioTEPA or 5-Fluorouracil in an attempt to see if they show a similar effect to that seen in the patient. ThioTEPA resulted in a marked regression whilst there was no effect from 5-Fluorouracil on tumour growth—confirming the ThioTEPA effect seen in the patient.

Many drugs are used in clinical practice to treat malignant ovarian disease with widely varying tumour response. Because of this unpredictable behaviour many investigators have been engaged in work trying to predict the response of individual tumours to various forms of therapy—especially chemotherapy. Among systems used are short-term monolayer tissue culture, organ culture and heterotransplantation—rendering the animal immunoincompetent. Therapy based on results from these systems has not been successful in altering the clinical course of the disease.

The mouse mutant *nude* (athymic) is a new and valuable tool in studying human tumours. Mice with this mutation lack a thymus (and so T lymphocytes) and are thus immunologically incompetent. Gaard & Povlsen (1, 2) have transplanted and studied several tumours in these mice and have demonstrated conclusively that the mice are unable to reject the foreign tissue. Further, after passaging, tumours have maintained their integrity both morphologically and biochemically. The importance of these mice, which need no irradiation or

chemical immunosuppression to act as host to tumour heterotransplantation in the research of various modalities of therapy is illustrated in this preliminary report of our experience of growing ovarian cancer and testing cytostatic drugs against it. One case is presented in detail.

MATERIALS AND METHOD

We obtained tumour tissue at laparotomy from patients suffering from ovarian cancer. Surgery was performed as a primary procedure with radiotherapy or chemotherapy to follow. Within an hour of surgery representative non-necrotic areas of the tumour were chopped into small pieces (2 mm diameter) and implanted subcutaneously in the mice. Tissue was also sent for light and electron microscopy. When the tumour had grown to 0.5–1 cm diameter it was transplanted into 30 mice ready for experimentation.

Mice

We used young females weighing 20 g *n/nu*/BALB/c/BOJ, stored on an air flow bench in a conventional mouse room at 28°C and fed standard pellets and tap water *ad lib*.

Drugs

The drugs tested have, in the first experiment, been restricted to those in clinical use in patients with ovarian cancer at the Norwegian Radium Hospital—ThioTEPA and 5-Fluoro-Uracil.

ThioTEPA (Tifosyl®) was obtained as a powder in 10 mg vials. This was dissolved in physiological saline for a regimen of 4 mg/kg/day intraperitoneally for 5 days with a two week pause between treatments.

5-Fluoro-Uracil (Fluoro-Uracil®) was obtained in ampoules containing 250 mg in 5 ml. This was diluted in physiological saline for a regimen of 25 mg/kg/day intramuscularly for 5 days, also with a two week pause.

The control group received 0.1 ml saline intraperitoneally in the same manner.

CONTROL

0.1 ml saline i.p. 5 □

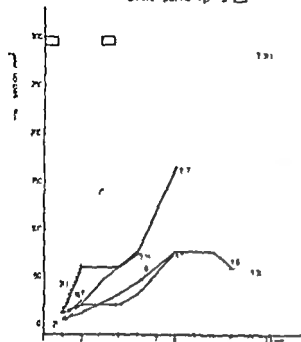


Fig 1

Assessment

The efficacy of therapy was assessed by measuring two diameters of the tumour weekly and calculating the cross section. A control of drug toxicity was made by weighing the animals weekly. At the conclusion of the second course of treatment mice from each group were sacrificed and tissue prepared for both light and electron microscopy.

Electron microscopy

Representative 1 mm cubes of tissue were fixed in 2% phosphate buffered glutaraldehyde, postfixed in osmium tetroxide, dehydrated through graded alcohols and embedded in EPOX 812 using propylene oxide as an intermedium. Semithin sections were made with an LKB Pyramitome equipped with glass knives and stained with toluidine blue for light microscopical orientation. Ultrathin sections were cut with glass knives mounted on an LKB Ultratome III, collected on naked copper grids, stained with uranyl acetate and lead citrate and examined in a Philips EM 701 electron microscope.

Case history

The patient whose tumour was studied was a 53 year old para 4 whose menopause at the age of 47 had been unremarkable. In September 1973 she had noticed increasing abdominal distension and intestinal colic. Three months later she consulted her doctor because of breathlessness and a 5 kg weight loss. He found ascites, a left sided pleural effusion and an abdominal tumour filling the whole pelvis extending up to the umbilicus and fixed to the pelvic wall. At laparotomy the peritoneal cavity was filled with

a tumour arising from the ovary. The omentum was fully infiltrated. A biopsy was taken and tissue was as described. Malignant cells were found in the peritoneal fluid and the case was classified as a stage IV cancer and treatment begun with ThioTEPA. She remained well on regular ThioTEPA until June 1974, she developed ascites and symptoms of a subacute obstruction. In view of her poor prognosis treatment was palliative. She died in August of bronchopneumonia, with widespread metastases from her ovarian cancer 6 months after initial therapy.

RESULTS

To date we have transplanted tissue from 10 tumours with successful growth in all except 3. These 3 failures were at the beginning of our experience and we feel that experience gained earlier now helps us in the selection of cancer tissue.

Initially there was a lag period of 3 to 4 months before the tumour showed appreciable growth following this growth rate was steady. In the second passage the rate was faster. In the case under review 20 mice received tumour pieces at the first passage subcutaneously and at 6 weeks 17 had visible tumours whilst 10 mice received a minced cell suspension and here at 6 weeks for 5 mice to develop a tumour of considerable size.

5 FLUORO URACIL

□ 25mg/kg/day x 51m

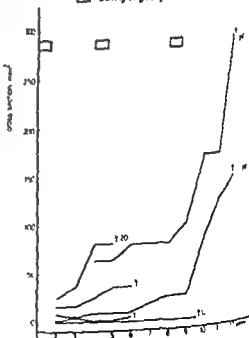
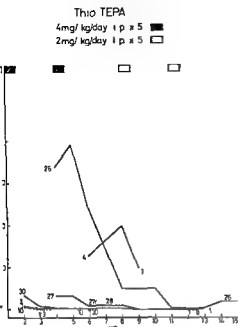


Fig 2



size Unfortunately not all the animals were healthy and several died of natural causes. The life span of these mice varies greatly. It has been found that they die of a wasting syndrome so it is very important that they be as uniform in size and health as possible at the beginning of the experiment.

Saline group

5 mice in this group received saline and their tumours continued to grow at a steady rate (Fig 1) and died after 4 weeks.

5 Fluoro-Uracil group

Of the 11 mice in this group one died in the third week and two in the seventh. Those surviving showed a continued steady growth of tumour (Fig 2).

ThioTEPA group

Despite careful toxicity studies beforehand 3 mice died before they received their second course thus the dose was halved from 4 mg to 2 mg/kg/day for 5 days. Tumour growth is shown in Fig 3. This study ended after only 2 courses as at this time one could see a clear difference in the growth patterns following the use of the two drugs and we wanted to sacrifice some mice for histology whilst there was still some tumour present in this last group.

Remaining ThioTEPA mouse

At the conclusion of the experiment one mouse was alive—with complete regression of the tumour. This mouse had received 4 mg/kg/day for 1 course and 2 mg/kg/day for 2 courses. Eight weeks after the cessation of the last course the tumour had grown almost as large as it had been at the beginning of the experiment. We gave a further 3 courses of ThioTEPA—see Fig 4—with obvious effect but less marked than the initial effect. After the third course the mouse appeared to be wasting so injections were stopped—and the mouse continued to thrive so we concluded that toxicity had played a part. Once more the tumour began to grow—but the mouse died before we could give further drug suddenly after 37.5 weeks.

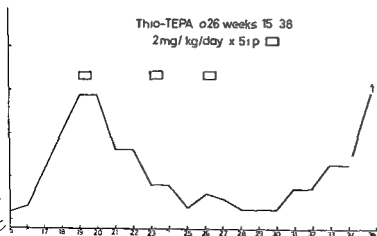


Fig 4

ECTOPIC PREGNANCIES AND THE USE OF INTRAUTERINE DEVICE AND LOW DOSE PROGESTOGEN CONTRACEPTION

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act In a series of 71 ectopic pregnancies ten were treated with the use of an intrauterine device (IUD) and with the use of low dose progestogen contraceptive or comparison a series of 800 legal abortions were performed and in 20 of these cases pregnancy originated an IUD was in utero and in 13 during the use of low contraceptive pills. When using the above mentioned contraceptive methods the possibilities of an ectopic pregnancy should be taken into consideration.

as early as 1929 Grafenberg reported (4) ectopic pregnancies in connection with the use of IUDs (intrauterine contraceptive devices) and it has been observed that pregnancies which occur during the use of IUDs show a greater tendency to be ectopic pregnancies which occur when IUDs are not

used (6). Recently attention has been also paid to the fact that ectopic pregnancies have occurred during the use of low dose progestogen contraception (1, 2, 3, 5). Because both of these contraceptive methods are widely used in our area and an ectopic pregnancy may be a dangerous condition we have in this study examined to what extent ectopic pregnancies treated in our hospital have originated during the use of these contraceptive methods.

MATERIAL

From 1972 to 1974 a total of 9016 mothers were delivered in the area in which this material was collected. During this period 71 ectopic pregnancies were treated surgically which corresponds to 7.8 ectopic pregnancies per 1000

1 Patients

Age (y)	Number of previous abortions	Number of previous deliveries	Period of use of contraceptive method (months)	The name of IUD or progestogen used	Endometrial histology	Result of immunological pregnancy test
38	—	3	72	Lippes Loop	—	Pos
31	1	1	36	Lippes Loop	Secretory	Neg
31	1	3	2	Lippes Loop	Secretory	Neg
35	—	1	36	Previgon	Decidual reaction	Neg
28	—	4	18	TCu 700	Secretory	Pos
35	—	2	3	Progestasert™65	—	Pos
74	—	2	18	Lippes Loop	—	Pos
35	1 (legal abortion)	3	60	Gravigard	—	Pos
76	—	11	2	TCu 700	—	Pos
38	1	2	14	TCu 700	Secretory	Neg
74	—	3	8	Lynestrenol	Proliferation	Neg
70	—	2	3	Norethisteron	Proliferation	Neg
35	—	3	6	Norethisteron	Decidual reaction	Neg

deliveries. Investigations were made to ascertain to what extent ectopic pregnancies originated during the use of IUDs or low dose progestogen contraception.

Furthermore, for comparison, all legal abortions carried out in 1973 were studied to find out how many of these pregnancies originated during the use of IUD or minipills (low dose progestogen contraception).

RESULTS AND COMMENTS

In Table I the patients with ectopic pregnancies associated with the use of IUD or low dose progestogen contraception from 1972 to 1974 are presented. The number of IUD users was 10 and the average age of these patients was 32 years. Only one of these patients had no previous pregnancies and among the others the number of previous pregnancies ranged from one to four. Three patients had previous spontaneous abortions and one had a previous legal abortion. The period of IUD use ranged from two months to six years, being on an average two years and two months. The IUD trademarks were as follows: four cases of Lippes Loop, three of Copper T (T Cu 200) and one of Prevgyn. Progestasert TM65 (ALZA's progesterone uterine therapeutic system) and Gravigrad. In four patients preoperative curettage showed secretory endometrium and four patients had negative results from immunological pregnancy tests.

There were only 3 confirmed users of low progestogen pills. The average age of these patients was 26 years and the average period of use was 6 months. One of these used Lynestrenol (0.5 mg) and two Norethisterone (0.3 mg).

During the year 1973 a total of 800 legal abortions were performed in the area in which this material was collected. In 20 of these cases pregnancy originated while an IUD was in the uterus. In addition 13 patients had regularly used low progestogen contraceptive pills. During the same year 28 cases of ectopic pregnancies were treated at Oulu University

Hospital and of these there were four patients with IUD in uterus and two patients were regular low progestogen contraceptive pills (one). Thus among the IUD users 13 pregnancies corresponded to 20 legal abortions and among the users of low progestogen contraceptive pills 2 pregnancies corresponded to 13 legal abortion patients.

Since we have observed 13 ectopic pregnancies connected with the use of IUD or progestogen contraception we agree with Hawkins that in patients of abdominal pain who use these contraceptive methods the possibility of an ectopic pregnancy should be taken into consideration. Also of importance are the facts that the material diagnostic expedients gave negative results in some cases and in two cases a dangerous situation was in the process of developing.

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VALUE OF EXTERNAL VERSION IN FETAL MALPRESENTATION IN COMBINATION WITH USE OF ULTRASOUND

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During 1969-1974 six hundred and forty nine external versions were attempted during the last trimester on others with fetal malpresentation. The procedure monitored with ultrasound in 1969-1974. Most of at (70.0%) were made during the 32nd-36th weeks. The success rate after one or more attempts was being lower (67.0%) in nulliparous than parous (84.6%). The incidence of breech presentation at decreased from 4.5 to 2.9% ($p < 0.001$). The most complication was one premature labor but the infant survived. The perinatal mortality was 2.0% and after delivery of abnormal infants 0.8%. The combination of external version and the use of ultrasound is a safe method, avoids hazardous vaginal breech delivery and is recommended in obstetric practice.

Perinatal mortality and morbidity rates associated with vaginal breech delivery have been documented repeatedly (5, 10, 12, 15, 17). During the fetal period only the major complications are noted, whilst minor damage may not be evident later as mental and motor sequelae (14, 16, 20). Routine use of abdominal delivery, although it reduces the fetal risks, may increase maternal morbidity and create potential hazards in subsequent pregnancies.

Uncomplicated external version obviates all risks associated with vaginal breech delivery, decreased the number of caesarean sections because of breech presentation. Since the year 1969 we attempted external versions in fetal malpresentations in utero. This study was initiated after the introduction of ultrasound equipment into obstetric practice in our hospital. Such approach is of value in diagnosis of fetal presentation, number as well as placental site. Our results over 6 years are summarized in the present report.

PATIENTS AND METHODS

From 1969 all maternity welfare units in our region were instructed to direct the mothers whose fetal presentation was not cephalic or was uncertain during the 32nd week or later to our specialized maternity unit for external fetal version. To the end of 1974 491 women with fetal malpresentation have been collected. This represents 2.6% of 18 783 singleton deliveries in our unit during the study period. The exact position of the fetus and placental site were detected in most cases (89.0%) with ultrasound (Kretztechnik 4100 MGS). A breech presentation was diagnosed in 95.9% and a transverse lie or combined malpresentation in 4.1%. 50.1% of patients were nulliparous.

The study was performed on an out-patient basis. After emptying the urinary bladder the mother rested on her back with partially flexed thighs. The breech was displaced upward from the maternal pelvis and laterally to the side of fetal back. The fetal head was pushed to the opposite side and downward by the other hand. The performance was gentle and any force, especially against the placental site, was avoided. No vaginal manipulation was allowed. Sometimes one must wait for up to 10-15 min before the fetus own movements complete the version. If the fetus could not be turned in this direction an attempt was made in the reverse direction. Cardiotocographic recording of fetal heart rate (FHR) and uterine contractions before and after the attempt was performed routinely. The success of version was confirmed by ultrasound if uncertain by palpation.

Attempts at version were made once in 378 cases, twice within 1-2 weeks interval in 73 cases, three times in 37 cases, four times in two cases and six times in one mother yielding a total of 649 attempts. The intramuscular administration of spasmolytic, analgesic or tocolytic drugs (diazepam 10 mg, pethidine 50 mg or isoxuprine 10 mg) was used in 13.9% of cases before the 1st attempt and in 33.3% of cases before the later ones. No anesthesia was ever given.

Absolute contraindications to version were total or partial placenta praevia, multiple pregnancy, rupture of membranes, labour and contracted pelvis necessitating caesarean

Table 1 Relationships between gestational age, attempt and result of version as well as occurrence of spontaneous reversion (to breech) or version (to vertex)

Weeks of pregnancy	Number of attempts	Successful result		Spontaneous reversion		Unsuccessful result		Spontaneous version	
		No.	%	No.	%	No.	%	No.	%
28	2	2	100	—	—	—	—	—	—
29	3	3	100	—	—	—	—	—	—
30	11	10	91	—	—	1	9	1	100
31	11	7	64	1	14	4	36	1	100
32	43	35	81	—	—	8	18	1	17
33	9	64	69	9	14	28	31	1	7
34	115	84	73	12	14	31	27	2	6
35	107	70	65	7	10	37	35	—	—
36	94	49	52	3	8	45	48	2	5
37	71	34	48	2	7	37	52	1	3
38	66	29	44	2	7	37	46	1	3
39	18	10	46	—	—	8	44	—	—
40	10	4	40	1	25	6	60	—	—
41	4	1	25	—	—	3	75	—	—
42	2	1	50	—	—	1	50	—	—
Total	649	403	62.1	37	9.2	246	37.9	11	3.3

ean section apart from the fetal presentation. Previous caesarean section or other uterine scar or anomaly were regarded as relative contraindications.

RESULTS

Incidence of breech presentation at birth

The incidence of breech presentation at birth was 4.3% among 15 279 singleton pregnancies prior to the study in 1965–1968 and during the study 2.9% among 18 783 parturients. The decrease of 1.6% is highly significant ($p < 0.001$).

Gestational age at version

All the attempts in relation to gestational age are given in Table 1. Most attempts (70.0%) were made during the 32nd–36th weeks. Twenty seven versions (4.2%) were tried before the 32nd week and six (0.9%) after the 40th week. The mean pregnancy duration during the 1st attempt was 34.8 weeks (range 28–42), during the 2nd 35.7 (range 31–38) and during the 3rd 37.0 (33–41).

Success rate

The success rate (per cent) in relation to gestational age is shown in Table 1. The mean was 62.1%. The 1st attempt was successful in 66.7% and the 2nd and 3rd in 43.6%. The final turning rate after one or more version in the series was 76.2% and was lower (67.0%) in nulliparous than parous women (84.6%) ($p < 0.001$). The rate of success decreased some

what with advancing gestational age. However, differences between various weeks (until 36) showed a small ($p < 0.05$) if any significant size (height or weight) of the mother and the success rate were not related. The various fetal sites: anterior (40.2%), posterior (46.1%) or wall (12.3%) or lower segment (1.4%) did not influence the version rate.

Spontaneous version

Spontaneous version occurred 40 times in 491 mothers (Table 1). After successful external version reversions to breech occurred in 33 mothers, i.e. 9.2% of all successful versions. Because of

Table 2 Complications in 491 mothers in relation with 649 successful or attempted external versions (% out of 649 versions)

Complication	No.	%
Uterine contractions	27	4.1
Uterine bleeding	3	0.4
Uterine bleeding and contractions	2	0.3
Variation in FHR (over <100 or >160/min)	3	0.4
Rupture of membranes and labour	1	0.1
Abruption of placenta	0	0
Fetal death	0	0
Total	36	5.5

III Duration of pregnancy and method of delivery in mothers with breech or vertex presentation at and overall

	Breech presentation (N=117)		Vertex presentation (N=374)		Overall (N=491)	
Duration of pregnancy						
before the 36th week	2	(1.7%)	2	(0.5%)	4	(0.8%)
during the 36-37th week	11	(9.4%)	10	(2.7%)	18	(3.7%)
after the 37th week	1	(0.9%)	3	(0.8%)	4	(0.8%)
Total	39	(33.0%)	40	(10.7%)	40	(8.2%)
Method of delivery						
Normal vaginal	87	(74.3%)	357	(95.4%)	439	(89.7%)
Cesarean section	35	(29.7%)	17	(4.5%)	52	(10.6%)

External version was repeated only 10 women with spontaneous reversion still had breech presentation at birth which is 2.5% of successful versions. Three attempts to vertex occurred after one unsuccessful attempt in nine cases, two attempts in three cases and three attempts in one case. The rate of successful external version was 5.3 per cent of all unsuccessful attempts. Of all spontaneous versions or reversions 13 (26%) occurred after the 35th week (16%) after the 36th week and four (8%) after the 37th week. One reversion was detected in utero at VI even during the 40th week.

Complications

Complications were mostly mild uterine contractions (Table II). In five cases slight uterine bleeding occurred but ceased within 1-3 days. In 11 cases the FHR varied significantly (<100 or >160/min) but returned to normal 8-14 hrs later. Healthy infants in vertex presentation were

born at term. In one case the membranes ruptured 12 hrs after successful version during the 34th week and the delivery took place two days later. The infant survived and was healthy. All the placentas were checked in order to detect signs of possible retroplacental haemorrhage aroused during version. A small organized clot was found in two cases. The occurrence of complications was not related to gestational age during the attempt, parity, placental site, result or number of attempts nor to maternal drug treatment before attempt.

Delivers

Although the mean duration of pregnancy did not differ in mothers with breech (N=117) (=unsuccessful version) or vertex (N=374) (=successful version) presentation at birth, the rates of preterm deliveries were slightly higher in breech than vertex presentation (Table III). Out of 491 mothers with initial fetal malpresentation in 82 cases (16.7%)

IV Distribution of Apgar scores and fetal deaths in relation to fetal presentation at birth

	Breech presentation (N=117)		Vertex presentation (N=374)		Overall (N=491)	
Apgar scores at 1 min						
3	5	(4.3%)	3	(0.8%)	4	(0.8%)
7	13	(11.1%)	7	(1.9%)	9	(1.8%)
10	81	(68.6%)	86	(23.0%)	95	(19.3%)
Fetal deaths						
Antenatal	2 (prolapse of cord anomaly in CNS)		3 (congenital heart disease anomaly in CNS multiple anomalies)		5	
During the 1st postpartum week	3 (pneumonia 21 hrs, sepsis and congenital heart disease aspiration)		2 (respiratory distress syndrome umbilical hernia)		5	

vaginal breech delivery occurred. Caesarean section was performed 52 times (10.3%) in the whole series. The frequency of section in breech was 30.0% and in vertex 4.5%. In 26 of 239 mothers with X-ray pelvimetry the true conjugate was less than 11.0 cm. In these circumstances routine caesarean section for breech presentation is mandatory in our department. After successful version 16 out of them delivered vaginally, thereby avoiding section.

Infant

The distribution of Apgar scores did not differ significantly according to fetal presentation at birth (Table IV). Four of five intrauterine fetal deaths occurring 3-7 weeks after the version or attempted version were caused by anomalies. In one case the cord was prolapsed during full term breech delivery and the infant died. In addition five fetuses, three born in breech and two in vertex, died during the 1st week of life. Thus the final perinatal mortality was 4.3% in breech and 1.3% in vertex presentation, being 2.0% in the whole series. After excluding the anomalies the mortality rate was 2.5% in breech and 0.3% in vertex presentation.

DISCUSSION

The role of prophylactic external version in modern obstetrics is obscure. Many authors have recommended it as a safe and simple method which lowers the incidence of breech presentation at birth (1, 6, 8, 9, 11, 13, 18). Conversely it has been stated that spontaneous versions and reversions make the policy of external version useless and only potentially dangerous to the fetus (2, 3, 7, 19).

As far as we know, our study is the first to combine the policy of external version and the use of ultrasound. The diagnosis of fetal presentation often is uncertain if based only on palpatory findings. With the aid of ultrasound we could safely confirm the presentation and exclude multiple pregnancies and the cases with placenta praevia when the version is potentially dangerous. Also during version it is of importance to know the placental site in order to avoid any force against it. The results summarized now have convinced us that the combination of ultrasound with external version is an advantage in obstetric practice.

A calculation of the value of external version is hampered by the impossibility to detect the cases in

which later spontaneous version might have occurred. In our series when the attempts were performed later than in previous series (1, 9) the final rate of spontaneous version in vertex failed attempts was 9.3%. This frequency probably has been greater without our experience but at this rate only 26 mothers would have had vertex presentation at birth instead of 33. The final efficacy of our policy was ascertained: decrease of 1.6% in the incidence of breech presentation at birth. Jokela (9) succeeded to lower incidence by about 1.0%, Bock (1) by 1.1%, Hibbard & Schuman (8) by 1.5% and MacLellan by 1.2%.

In our hands external version was safe. The serious complication was one premature labour which was quite probably initiated by a spontaneous version during the 34th week. The information that the placental site must contribute to the late serious accidents during attempt. We could not exclude the cases with contraindications such as with placenta praevia. In the 860 cases of Jokela (13) two infants died because of placenta praevia which started to bleed after external version. Possible fetal hazards include cord complications. Jokela (9) lost one and Hibbard & Schuman (8) two infants because of cord strangulation. Our only accident was one prolapse of the cord which occurred during full term breech delivery in a mother who had experienced one unsuccessful version two weeks previously. We may assume that this accident would not have happened if we had managed to turn the fetus. No abruptio placentae took place. Abruptio occurred in two of 314 patients of Ellis (4) who performed versions under anaesthesia and in three out of 866 patients of Bradley & Watson (2) who did not use anaesthesia. As usually in breech presentation fetal anomalies were common but even in the presence of them the perinatal mortality of 2.0% in the whole series was lower than the mean of 2.3% in our unit during the study period. If the anomalous infants were excluded the perinatal mortality rate was only 1.3% in mothers with successful versions and 0.3% in mothers with unsuccessful versions and breech presentation at birth.

In conclusion we recommend the gentle prophylactic version in fetal malpresentation from utero to clinical practice. The procedure combined with preceding ultrasound examination

ate of success or complication is not so strictly related to the gestational age that version must be at any given time during the last trimester of pregnancy. Mild premedication with spasmolytic or analgesic drugs can help and does not cause any harmful side effects.

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CHORIONEPITHELIOSIS A RARE BENIGN TROPHOBLASTIC DISEASE

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act Chorionepitheliosis a benign trophoblastic disease is a rare entity since only 76 cases have been shed The diagnosis was made in two instances andoscopic biological and clinical data are presented lesion must be distinguished from syncytial endometritis chorionadenoma destruens and chorioncarcinoma The possible histogenesis is discussed

Chorionepitheliosis is a benign trophoblastic invasion of the uterus which penetrates deeper than in case of the so called syncytial endometritis In other words it seems to occur when the local defence mechanisms against the invasive potential of trophoblast cells of the placental bed are overwhelmed The report of a case by Schopper & Phliss with discussion of 25 other cases in the literature no other case seems to have been published This paper deals with two new cases and with the differential diagnosis of the disease

CASE REPORT

Case

A 35 year old caucasian woman attended the gynaecological clinic for the first time at the beginning of her pregnancy Spontaneous abortion had occurred at 6 weeks in her first pregnancy and was not followed by a second pregnancy The second pregnancy was terminated by a caesarean section at 42 weeks after an unsuccessful trial of labour giving birth to a 4.2 kg healthy boy At the first delivery the patient's weight was 73.5 kg (height 162 cm) Laboratory data were unremarkable Urine and blood were negative PBI 10.4 gamma⁶⁷ Rubella and Toxoplasma antibodies were negative Because of excessive birth weight and a family history of diabetes (mother and brother) a glucose tolerance test was

performed values were in the normal range (0.72-1.11-0.61) During pregnancy blood pressure remained between 115-135 and 80-85 mm Mercury weight gain was in normal limits (6.5 kg) Neither glucose nor protein appeared in the urine

Because of the previous caesarian sections and the patient's request for tubal ligation a planned caesarean section was performed at 39 weeks of pregnancy Delivery was easy and gave birth to a healthy boy 3.75 kg in weight

In spite of the administration of ergonovine 0.4 mg and ergometrine 0.15 mg the uterus remained atonic After 10 minutes of observation ecchymoses appeared diffusely on the serosal surface of the uterus the obstetrician decided to perform a total hysterectomy no blood coagulation defect having appeared The immediate post partum progress was normal At the post natal examination 8 weeks after delivery the abdominal and vaginal scars had healed very well Slight oestrogenic activity was revealed in a vaginal smear general examination disclosed no abnormality Actually the patient is alive and well about a year after the diagnosis was made

The hysterectomy specimen presented a low transverse incision in the lower segment The uterine diameter was 10 cm weight was 1400 g The serosa was slightly elevated by brown haemorrhagic patches

Many blocks were cut involving the placental bed and the serosal patches Placental bed sections revealed occasional placental villi enmeshed in blood clots the underlying myometrium was slightly invaded by normal appearing placental bed giant cells The serosal lining was loosened by fresh extravasated blood and trophoblastic aggregates Some trophoblastic cells were located between underlying smooth muscle fibres Neither placental villi nor necrosis could be observed Abnormal migrating trophoblast consisted of cytotrophoblastic and syncytiotrophoblastic looking nuclei nor mitoses (Figs 1 and 2)

Second case

Mrs G B a 29 year old caucasian woman delivered spontaneously in 1969 a healthy full term boy weighing 2.8 kg One year later she aborted spontaneously Six months

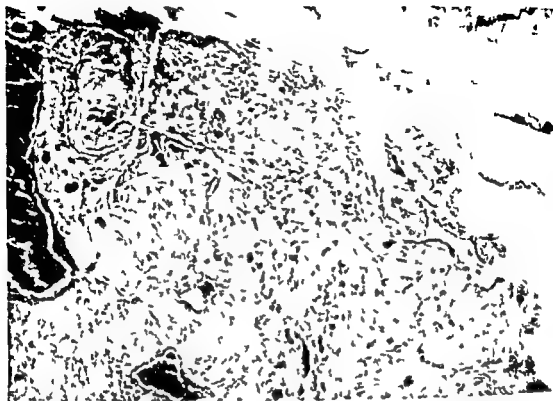


Fig. 1 Tissue section involving the serosal side of the uterine wall (H & E low power view 4×10). Sub-serosal haemorrhage is seen at the top; cyto- and syn-

cyto-trophoblastic cells are scattered between muscle fibres.

later she was operated upon for an unruptured left tubal nancy; the proximal end of the tube was resected. A cornual reimplantation of the tube was performed. Post-operative hystero-salpingogram showed two patent tubes but the patient complained of secondary dysmenorrhea. Ten months after salpingoplasty laparoscopy disclosed foci of endometriosis in the left uterosacral ligament. Since the patency of the left tube was doubtful by hydrotubation a left salpingectomy was performed without excision of the interstitial portion. Pathological examination showed tubal endometriosis. After a normal pregnancy the patient delivered spontaneously. Because of prolonged retention of the placenta a manual removal was attempted but the placenta had escaped into the abdomen through a left cornual rupture. Laparotomy was performed in order to remove the placenta; the left cornu was resected. Two samples of blood were obtained for gonadotrophin assay; results were in the normal range. Six months later the patient was menstruating regularly; gynecological examination was normal.

The pathological specimen consisted of a circular tissue fragment brownish in colour. Microscopic examination (Fig. 1) revealed definite cyto- and syncytiotrophoblastic cellular elements interspersed between smooth muscle fibres reaching the subserosal areas of the uterine wall. The picture was thus similar to that of the first described case.

DISCUSSION

The two reported cases showed features compatible with the diagnosis of chorionepitheliosis with a clinically benign chorionic invasion of myometrium. (1) Absence of necrosis, placental villi and vascular invasion are important diagnostic features. (2) Chorionepitheliosis has to be distinguished from syncytial endometritis, chorioma destruens and choriocarcinoma. According to Saphir (8) syncytial endometritis is not a true tumour; it denotes a condition in which there is a trophoblastic proliferation than normal combined with secondary inflammation. The trophoblastic infiltration is thus quantitatively more pronounced than in normal pregnancy so that endometrial myometrium are infiltrated by trophoblastic cells. (3) Boyd (2) stated that syncytial endometritis is a tumour in which the structure is benign with a corresponding absence of blood vessel invasion. Absence of haemorrhage and necrosis are important features in distinguishing syncytial endometritis from choriocarcinoma. The distinction

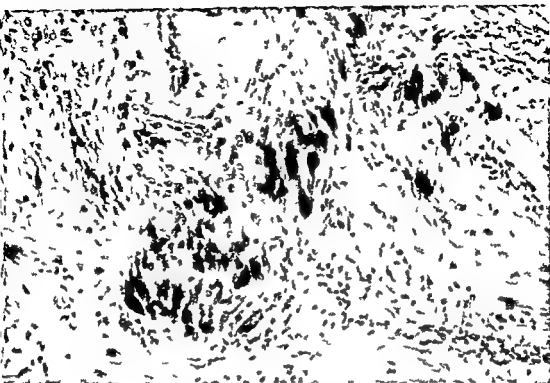


Fig. 1. High power view (H & E 10x10) of the same showing details of the myometrial invasion by blastocells.

■ of chorioadenoma destruens as listed by Kirk & Seah (6, 7) are excessive trophoblastic growth and penetration of trophoblastic elements (including whole villi) into the depths of the myometrium, sometimes involving the peritoneum, adjacent parametrium or vaginal vault. Blood vessels may be invaded by villi and associated trophoblast (4).

The picture in choriocarcinoma is that of columns

and sheets of trophoblast penetrating muscle and blood vessels in wild disorganization interspersed with clotted blood and necrosis with complete absence of a villous pattern. Necrosis of muscle is common (8). The diagnosis is justified only when the microscopic pattern, the biological (high levels of gonadotrophins) and the clinical (persistent bleeding, subinvolution of the uterus) behavior of the tumour suggest it is malignant.

Table 1. Differential diagnosis of trophoblastic diseases

	Syncytial endometritis	Chorioadenoma destruens	Choriocarcinoma	Chorionepitheliosis
horrhage	-	+	+	+
osis	+/- (decidua)	+	+	-
hoblast	+	+	+	+
d vessel	-	+	-	-
ision		(villi and trophoblast)	(trophoblast only)	

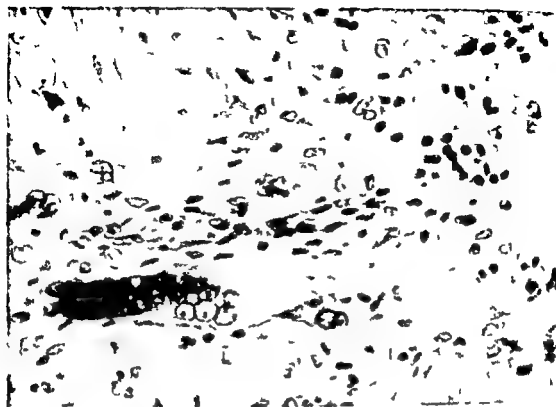


Fig. 3 Sub-serosal myometrium: smooth-muscle fibres are pushed away by multi-nucleated syncytiotrophoblastic cells (H. & E. 40 \times 10).

Chorionepitheliosis is a clinically benign chronic invasion of the myometrium (3). Absence of necrosis, placental villi and vascular invasion are important distinguishing features. Table 1 summarizes the most important pathological findings characteristic of the four conditions in which the invasive potential of the trophoblast is more pronounced than in normal pregnancy.

From the cumulative data it appears that chorionepitheliosis shares features that are nearest to that of syncytial endometritis: the depth of trophoblast invasion is more pronounced in chorionepitheliosis.

Since the histo-pathological characteristics of trophoblastic diseases are rather restrictive and the behaviour of trophoblastic diseases is difficult to predict on microscopic observation alone, definite diagnosis would imply an accurate clinical and biological follow-up. Thus, chorionepitheliosis would be considered as a retrospective diagnosis (1).

More than 20 years have elapsed since C. Plouss (9) writing: "More study is necessary to answer to those who deny the existence of invasion and to investigate questions regarding the considerable variations in the placental site."

The true incidence of chorionepitheliosis is difficult to assess since it can be diagnosed only at caesarean section or caesarean-hysterectomy. In the two described cases it occurred in cases where therefore it could be possible that the normal defensive reaction against trophoblastic invasion was not acting normally. The absence of de gonadotrophin titres and the benign evolution of the disease lend support to the thesis of a benign although excessive trophoblastic invasion.

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SHORT COMMUNICATION

MEFENAMIC ACID IN DYSMENORRHEA

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etiological etiology for dysmenorrhea is stressed by several gynecologists (2, 9, 10), especially since they have discovered that prostaglandins (PGs) control the activity of the uterus. The antiprostaglandins are promising for the treatment of dysmenorrhea (8, 9, 10). Salicylates are less potent inhibitors of prostaglandin synthetase than mefenamic acid, which also inhibits the binding of PGs to the cell (6, 11). For these reasons, mefenamic acid might be effective in the treatment of a dysmenorrheic patient. This study was designed to observe the clinical symptoms, uterine activity and the primary plasma prostaglandins (E & F) and progesterone (P) before and after the treatment of a dysmenorrheic patient with mefenamic acid (Ponstan®).

Eight patients had severe dysmenorrhea which rendered them unable to work during their 1st day of menstrual bleeding. All patients had used other analgesics before this treatment with no complete relief. They had no pathology on gynecological examination except for possible uterine hypotonia.

All patients received 750 mg single dose of mefenamic acid during their 1st day of menstruation. The intrauterine pressure (IUP) was measured by the microballoon method during the 30 min period before and the 3 hours period after the medication (4). Plasma PGE, PGF and P were determined (by RIA (1, 5)) before and 3 hours after the medication. Their dysmenorrheic symptoms were recorded during the four hour experimental period. Uterine resting pressure, frequency of contractions and also slightly the active pressure decreased in about 2 hrs after mefenamic acid (Fig. 1). Subjective relief of dysmenorrheic pain was coincident with the decrease in uterine activity. One patient taking oral contraceptives without help for her

dysmenorrhea had no further decrease in IUP and no relief of pain when treated with mefenamic acid.

The plasma PGE was 39 ± 6 pg/mg before the medication and 32 ± 5 pg/ml 3 hours after 750 mg single dose of mefenamic acid. Respective values for PGF were 9 ± 2 and 5 ± 1 pg/ml in 10 successfully determined pairs of plasma samples. Because of variation in plasma PG determinations, the statistically nearly significant decrease ($p < 0.05$) in plasma PGF with mefenamic acid remains slightly uncer-

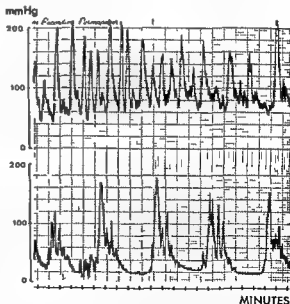


Fig. 1 Uterine activity before (upper line) and after a single dose of 750 mg mefenamic acid during the 1st day of menstruation. Original recording. Note the decrease in uterine resting pressure and frequency of contractions nearly unchanged active pressure (decreasing later on) coincident with the disappearance of dysmenorrheic pain.

tain. Plasma P was 2.9 ± 0.16 and resp. 2.4 ± 0.17 ng/ml. There was no significant change in plasma PG/P ratio, a far more important factor than PG or P alone (3). The primary levels of plasma PGE & F in dysmenorrheic patients were also in the same range as in the nonpregnant patients studied for other purposes in our laboratory (for PGF $15-80$ pg/ml for PGF $5-30$ pg/ml). Nor have Wilks et al. (11) given any diagnostic value on plasma PGs in establishing the cause of dysmenorrhea. Hyperprostaglandinemia can exist in patients with dysmenorrhea associated with vomiting, diarrhea and pyrexia (7) — a possible inborn error of metabolism with deficiency of 15-hydroxyprostaglandin dehydrogenase.

26 study patients received 500 mg mefenamic acid every 6–8 hours. The medication was started as soon as they realized the menstrual pains were appearing. Out of 75 treated cycles, 67 cycles became painless, in 4 cycles mild dysmenorrheic pains remained, and in 4 cycles of 4 patients there was no relief of pain.

Two separate gynecologists used the same treatment for about 200 cycles of 50 dysmenorrheic university students and reported about 90 per cent of the cycles to have turned painless.

Mefenamic acid decreases the uterine activity of a dysmenorrheic patient and relieves the pain so frequently that it can be used clinically in the treatment of true dysmenorrhea. Double-blind study (with placebo) is required for final conclusion of clinical efficacy.

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Letter to the Editor

Sir

Your article on ectopic pregnancy Dr Motoi Saito (*Acta Obstet Gynecol Scand* 54: 227 1975) on the basis of a basal body temperature record make the following statement: "The result seems to offer support for the postulation by Iffy that delayed ovulation is the main cause of tubal gestation and it is necessary to suggest that this conclusion is an error on the part of the authors" and the editors of the journal "with regard to the interpretation of the BBT chart presented in Fig. 1 of the paper quoted: 'In evaluating the temperature curve the authors state that ovulation occurred on day 19 of the intermenstrual cycle. However, in actual fact, on the 19th day the temperature was only 36.5°C following about 10 recordings between 36.4 and 36.5°C during the follicular phase. It was only after that the temperature elevation became significant, reaching a peak of 37.1°C on the 27th day of the cycle, after a gradual rise. This pattern called 'stepwise' temperature rise is well known to infertility work and reproductive biologists (1-3, 7). It is their general consensus that ovulation may occur almost any point of the ascending slope and that the luteal phase belongs to the category of 'luteal phase defect' (2), a recognized cause of infertility and reproductive abnormalities (6). On the ground of this knowledge I feel it justified to propose that in the case under discussion the delayed ovulation occurred sometime between the 20th and 27th days of the cycle. Accordingly it follows that the authors' conclusion is more than refuting it. Dr Saito and his colleagues should add one more to the several literary BBT records where the occurrence of delayed ovulation

and subsequent menstruation provided substantial evidence on behalf of the menstrual reflux theory (4) of the etiology of ectopic gestation.

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HISTAMINE METABOLISM IN NORMAL PREGNANCY AND IN TOXAEMIA OF PREGNANCY

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act The urinary excretion of histamine and its metabolites methylhistamine methylimidazoleacetic acid imidazoleacetic acid was measured under standardized dietary conditions in 24 women with normal pregnancy and in eleven patients with toxemia of pregnancy. In addition histamine metabolism was studied in healthy women at delivery and in four other healthy pregnant women during treatment with aminoguanidine. It is an inhibitor of diamine oxidase (histaminase). A decrease in the urinary excretion of methylimidazoleacetic acid was observed in normal pregnancy as well as toxemia of pregnancy compared to non pregnant women. In two toxemic patients and in one of the healthy women the urinary excretion of unmetabolized histamine moderately increased. Despite the very high diamine oxidase activity in the plasma and in the uterus during pregnancy there were no signs of altered catabolism of exogenous histamine in the pregnant women. Smoking decreased the urinary excretion of the quantitatively dominant histamine metabolite methylimidazoleacetic acid in pregnant subjects as it also does in nonpregnant subjects. The necessity of standardized dietary conditions in study of histamine metabolism in man was again emphasized.

rapid formation of histamine is seen during pregnancy in the rat, mouse and hamster (12, 19). In human pregnancy the high histaminolytic activity in maternal plasma and the placenta may also indicate a connection between histamine metabolism and the pregnant state. However, the evidence for an increased production of histamine in human pregnancy is so far less convincing than in the species mentioned above. Human fetal tissues can form histamine from histidine *in vitro* (14) but the rate of formation is very slow compared to rat and mouse tissues. Measurements of blood and plasma histamine have given some evidence of histamine formation in the human fetus (for references see 17)

and several authors (1, 2, 4, 5, 11) have reported increased values of urinary histamine in pregnant women.

During the last few years methods for the determination of the main metabolites of histamine in urine have become available. These are methylhistamine (MeHi), methylimidazoleacetic acid (MeImAA) and imidazoleacetic acid (ImAA), the latter being the end product of the action of diamine oxidase (DAO) or histaminase on histamine. In this investigation the urinary excretion of histamine and its metabolites was measured in normal human pregnancy and in toxemia of pregnancy as abnormalities in histamine metabolism have been reported in this disorder (17).

PATIENTS AND METHODS

The urine was collected for 24 hours in twenty-four healthy pregnant women with normal pregnancies (age 23-40 years, parity I-IV) and in eleven patients with toxemia of pregnancy (age 23-37, parity I-III). The duration of the pregnancy at the time of the urinary examination varied between six and forty weeks. Some of the subjects were studied twice during the course of pregnancy. Four other healthy pregnant women were going to have legal abortions (age 18-47, parity I-IV). In three of them the urine was collected on two consecutive days, the second day during aminoguanidine treatment (aminoguanidine sulphate 1 mg/kg body weight by mouth every six hours (8)). The fourth woman was given an intravenous injection of ³H histamine during aminoguanidine treatment. In addition in five other healthy women (age 21-33, parity I-III) the urine voided at the first micturition after delivery was collected. All subjects making a complete 24-hour urine collection period ate a standardized diet as described by Granerus (6).

The urine was acidified with hydrochloric acid and was kept cold until analysis. All patients were screened for bacteriuria three times during the pregnancy and were

Table 1 Urinary excretion of histamine (Hi) methylhistamine (MeHi) methylimido oleacetic acid (MeImAA) and imido oleacetic acid (ImAA) in normal pregnancy, toxæmia of pregnancy and a series (6) mean values and ranges ($\mu\text{g}/24$ hrs)

	Number of subjects (samples)	Hi	MeHi	MeImAA	ImAA
Healthy pregnant women					
Total	24 (78)	45 (13-190)	210 (90-360)	3 100 (2 100-6 000)	3 100 (400-5 800)
Smoking	9 (11)	50 (13-190)	230 (90-370)	3 600 (2 100-6 000)	3 300 (1 700-)
Non-smoking	16 (17)	43 (19-180)	200 (170-360)	2 900 (1 400-3 800)	3 000 (400-5 800)
Toxæmia of pregnancy (non smoking)	9 (10)	54 (14-220)	210 (170-360)	2 800 (1 700-4 000)	2 700 (1 700-)
Non pregnant women (non-smoking)	8 (8)	23 (6-46)	160 (60-350)	2 200 (1 700-3 500)	

regularly controlled for albuminuria. None of the subjects showed any sign of urinary tract infection in connection with urine collection. In the toxæmic group all women were healthy at the beginning of pregnancy.

The diagnosis of toxæmia was based on an increasing blood pressure, rapid weight gain and/or peripheral oedema and proteinuria according to the criteria of the American Committee on Maternal Welfare. Three patients had severe preeclampsia (see Table V) with a systolic blood pressure of 160 mmHg or more and/or a diastolic blood pressure of 110 mmHg or more measured on two occasions at intervals of six hours and albuminuria of more than 5 g per day. The patients were treated in hospital with rest, diuretics and antihypertensive drugs. The are listed in Table V. Besides these drugs all subjects received prophylactic iron and vitamin therapy.

Urinary analyses

Histamine (Hi) was determined by bio-assay on the isolated guinea pig ileum after absorption of histamine onto a strongly acid cation exchange resin as described by Wetterqvist & White (22).

Methylhistamine 1-methyl-4-(β -aminoethyl)-imidazole (MeHi) was determined by spectrophotometric quantitation of the methylhistamine complex with 2,4-dinitrofluorobenzene (DNFB). The method has been described by White (23) and Granerus et al (8). In 67 determinations a mean recovery of 83% (S.E.M. $\pm 5.5\%$) was obtained and was corrected for.

1-methyl-4-imido oleacetic acid (MeImAA) was converted to the MeImAA ethyl ester which was extracted with ether. The final separation and estimation was done by thin layer chromatography. Every urine sample was analyzed twice and the mean value was corrected for losses during the analysis. The standard deviation of a single determination was found to be 0.76 mg as calculated from 47 duplicates with a mean value of 3.20 mg. A detailed description of the method has been given by Granerus & Magnusson (7) and Granerus (6).

4-imido oleacetic acid (ImAA) was measured by a method similar to the MeImAA method with an initial hydrolysis of conjugated ImAA and spectrophotometric quantitation of the ImAA with diazotized sulfanilic acid. Usually 100 ml of urine was evaporated to dryness on the steam bath. The residue was taken up in 6 ml of 2 mol/l HCl and hydrolyzed at 145 °C. The hydrolysate was refluxed for 1 h in 10 ml of 1 mol/l HCl in absolute ethanol to convert to the ethyl ester. The ester was extracted with saturated ether. The first 10 ml of the eluate and the next 40 ml were collected and cautiously dried. The residue was then transferred to a flask with 3+2 ml 0.1 mol/l HCl. Complete hydrolysis of ImAA ester was achieved by evaporation on the steam bath. The ImAA was subjected to chromatography on cellulose-coated plates (MN 300). The plates were developed in *n*-butanol/acid/water (4:1:1) until the front had migrated 10 cm. ImAA HCl (Koch Light) was used as standard substance. After drying at 100 °C the chromatograms were sprayed with 0.1% diazotized sulfanilic acid solution in 2.5% sodium carbonate solution. The red spots of ImAA derivative which had an *R_F* value of about 0.5 were scraped off the plates and eluted for one h with tert. butanol/water/10% sodium carbonate (25:25:5) as described by Hanson (10). The intensity was read at 500 nm in a Beckman DU photometer. The ImAA references from the chromatograms were used as standards and treated in the same way. Fifteen urine samples were analyzed in duplicate with addition of 500 μg ImAA (hydrochloride). The mean recovery of ImAA added to the urine was 70% (S.E.M. $\pm 3.0\%$). Correction of all ImAA was done with this mean recovery.

Determinations of ^{14}C labelled histamine and metabolites were carried out using the isotope dilution technique described by Schayer and described by e.g. Nilsson

I Urinary excretion of histamine (Hi) methylhistamine (MeHi) methylimidazoleacetic acid (IA) and imidazoleacetic acid (ImAA) in three pregnant women before and during treatment with guanidine

n values in $\mu\text{g}/24 \text{ hrs}$

Week	Before aminoguanidine (day I)				With aminoguanidine (day 7)			
	Hi	MeHi	MeImAA	ImAA	Hi	MeHi	MeImAA	ImAA
II	10	220	2 100	800	11	390	2 300	900
13	76	260	2 400	1 300	28	770	3 100	1 700
18	66	190	2 900	1 000	51	280	2 600	700

Lindell et al (16) The excretion of total ^{14}C in urine was measured as described by Schayer & (20)

RESULTS

maternal pregnancy

values and ranges of the urinary excretion of the MeHi, MeImAA and ImAA are given in Table I. In one of the healthy smokers a rather high value (190 μg) was found and this was repeated on two different occasions two weeks the last time after smoking had been withheld for a few days (180 μg). The only abnormally high value of MeImAA (5.8 and 6.0 mg) were also repeated in two smokers. Dividing the material into smokers and non smokers disclosed a significantly higher mean value of MeImAA in the smoking pregnant women $3.6 \pm 0.4 \text{ mg}/24 \text{ h}$ against $2.9 \pm 0.1 \text{ mg}$ (mean $\pm \text{S.E.M.}$) in the non smoking pregnant women ($p < 0.05$) (Student's *t* test). The urinary excretion of the main histamine metabolite ImAA was higher than and significantly different ($p < 0.005$) from the mean value of MeImAA in the non smoking and non pregnant women

(6). The mean values for histamine and MeHi were also somewhat higher but there was no significant difference between pregnant and non pregnant women. Neither histamine nor any of its metabolites were excreted in significantly greater amounts in the last trimester compared to the first and second trimesters.

In the three healthy pregnant women in whom DAO was inhibited with aminoguanidine a substantial increase was found in MeHi but no decrease in ImAA (Table II). Subject ME may be regarded as a control as the DAO activity has hardly been raised so early in the pregnancy. The efficiency of aminoguanidine in inhibiting the DAO activity during pregnancy was tested in one subject given ^{14}C histamine. A urine sample from an earlier experiment by Lindberg & Tornqvist (15) was available for analysis of the radioactive histamine metabolites excreted in the urine. The results are shown in Table III and for comparison the mean excretion values of radioactive histamine metabolites in four pregnant women without aminoguanidine treatment (from reference 18) are given. It can be seen that aminoguanidine almost abolishes the formation of ^{14}C ImAA in pregnant subjects also. There is also a

III Urinary excretion of radioactive histamine and its metabolites in a healthy pregnant woman given a ^{14}C histamine infusion during treatment with aminoguanidine

For comparison the mean values of histamine and metabolites in four healthy pregnant women injected with ^{14}C histamine subcutaneously and not treated with aminoguanidine are given below (data from reference 18). Excretion in per cent of excreted radioactivity in 12 hrs. Total ^{14}C in per cent of given dose

Week	Without aminoguanidine					With aminoguanidine				
	^{14}C Hi	^{14}C MeHi	^{14}C MeImAA	^{14}C ImAA	Tot ^{14}C	^{14}C Hi	^{14}C MeHi	^{14}C MeImAA	^{14}C ImAA	Tot ^{14}C
7	—	—	—	—	—	7	18	68	2	86
16-17	—	2	37	79	78	—	—	—	—	—

Table IV Excretion of histamine (Hi) methylhistamine (MeHi) and methylmida oleacetic acid (MeImAA) in the urine first voided after delivery (μg)

Subject	Duration of urine collection period (hours)	Urine volume (ml)	Hi	MeHi	MeImAA	Remarks
U S	7	230	0	20	400	
L A	7	475	7	70	1 300	Oxytocin*
I J	7.5	890	18	130	2 400	Oxytocin Diet not contr
A L	9	355	11	70	2 300	Oxytocin Diet not contr
B L	8	455	11	70	1 000	Diet not contr

* 1 ml Syntocinon (Sandoz)

corresponding increase in the labelled metabolites MeHi and MeImAA compared to the pregnant women without aminoguanidine treatment

B Parturition

The urinary excretion of histamine MeHi and MeImAA at delivery is shown in Table IV. Urine was collected from the beginning of the delivery i.e. about half an hour before the child was born except in subject I J where the urine collection period started 5.5 h before the time of birth and covered the whole length of labour. Oxytocin was given to subject L A post partum and in subject I J and A L it was given in order to induce parturition. Although urine was collected for part of a day only and the diet was not controlled in all subjects there were no signs of altered histamine metabolism at parturition.

C Toxaemia of pregnancy

Individual values of histamine and metabolites given in Table V together with drugs and clinical data. In Table I mean values are summarized for the non-smoking pregnant and non pregnant healthy subjects. Nine non smoking subjects with toxæmia. No significant difference was found in urinary excretion of histamine metabolites in healthy pregnant women and in toxæmia. The mean value of unchanged histamine was somewhat higher in the toxæmia subjects M K and Ma P. Subject M K had severe toxæmia and this was the only case in which the fetus died. Subject Ma P had a slight toxæmia with normal laboratory findings.

Table V Urinary excretion of histamine (Hi) methylhistamine (MeHi) methylmida oleacetic acid (MeImAA) and munda oleacetic acid (ImAA) in eleven pregnant women with toxæmia of pregnancy

Excretion values in $\mu\text{g}/24 \text{ hrs}$. A hydralazine (Apresolin) C chlorthalidate (Chlotride) Co xanthinol nicotinate (planon) L furosemide (Lasix) S sulphonamides

Subject	Week	Hi	MeHi	MeImAA	ImAA	Severity of toxæmia	Drugs	Remarks
I S (1)	31	38	210	2 000	1 700	Mild	C+A	uc neg Oestrol normal
M K	32	220	230	3 200	2 100	Severe	C+A+S	uc neg Oestrol normal
I S (2)	33	44	360	1 700	1 400	Mild	C+A	uc neg Oestrol normal
S A	33	14	210	2 400	1 500	Severe	C+A+Co	uc neg Duplex
E S'	33	55	160	4 000	3 000	Mild		
S L	35	77	230	3 100	5 600	Mild	C	uc neg
A M P	38	22	140	3 400	2 700	Mild	C	uc neg Oestrol normal
Ma P	38	110	310	4 000	5 400	Mild	C+A+L	uc neg
G B K	38	15	270	1 900	2 800	Mild	C+A	Oestrol normal
E O	39	21	120	2 800	3 100	Mild	C+A	uc neg Oestrol normal
U B A	39	22	120	3 300	3 400	Severe	C+A	uc neg
H U	40	29	170	2 900	3 600	Mild	C+A	

Cigarette smoker

* uc urine culture

DISCUSSION

on and his group have assumed that an increased histamine formation during pregnancy is in some way related to rapid tissue growth (for a review see Kahlson & Rosengren (12)). A markedly increased histamine formation was first found in the liver of the rat later in the mouse and guinea pig but not in the fetus of the rabbit or cat. The pregnant mouse also developed a high histamine excretion capacity in the kidney and the hamster in the placenta (19). In the present study it has not been possible to demonstrate any substantial increase in the histamine turn over rate during human pregnancy or at parturition although the measure included histamine and all its quantitatively important metabolites excreted in the urine. The mean excretion of MeImAA, the quantitatively most important histamine metabolite in man, somewhat greater in the pregnant women compared with the non pregnant women in the study reported by Granerus (6). Both these groups ate the same standardized diet which is necessary to keep the intake of histamine and bacteria from the food at a constant level (6). However, the pregnant woman may have a greater tendency for bacterial fermentation of histamine in the gastrointestinal tract and are therefore not so easily controlled by the diet as the non pregnant subjects. Unpublished observations by Granerus, Lindell and Westling in healthy pregnant women not on a standardized diet showed a steady increase in the urinary excretion of MeImAA in three of the four subjects. A value obtained at the beginning of the last trimester was normal in all subjects. Thereafter a sharp increase occurred to a level of 6-7, 6-9 and 15-18 mg/24 hrs in three of the subjects. In one subject a value of 35 mg/24 hrs was recorded on the day before delivery. After delivery a gradual decline occurred but rather high values were seen two weeks after delivery in one subject. It seems that these sometimes very high values of MeImAA without a standardized diet regimen are probably caused by a contribution of exogenous histamine of dietary or bacterial origin. Other reports have shown a definite tendency towards higher urinary histamine values in the third trimester (2, 5, 11), more pronounced in man. Histamine has been measured with chemical methods and the histamine values are generally lower. The resemblance between the rising histamine values during pregnancy and the increasing excretion of MeImAA without the standardized diet

may indicate that at least some of the histamine measured was coming from exogenous sources.

The highest values of MeImAA in this report and in the subjects studied by Granerus (6) consistently belonged to the smokers. In smokers without the standardized diet used here still higher and more varying excretion of MeImAA has been reported (21). However, it has been observed that after two or three days with the standardized diet the urinary excretion of MeImAA became normal despite continued smoking (Ekman & Granerus, unpublished results). Therefore, the larger amounts of histamine metabolites often encountered among smokers may not reflect an increase in the endogenous histamine turn over.

High values of ImAA in urine have been reported in patients with recurrent or inevitable abortion but not in toxæmia of pregnancy (9). The values obtained here were of the same magnitude in normal and in toxæmic pregnancy and were not different from the ImAA values previously reported in healthy males by Duner et al. (3). These authors pointed out that determinations of ImAA in man cannot be used clinically to estimate endogenous histamine formation as the ImAA excretion was significantly increased after histidine intake by mouth. Inhibition of DAO in three of the pregnant women did not cause any decrease in the amount of ImAA excreted which indicates that in pregnancy too ImAA is mainly derived from histidine and is not a quantitatively important histamine metabolite.

The excretion of unmetabolized histamine was also unaffected by aminoguanidine treatment as was previously reported in a study in five pregnant women (2) and the pattern of histamine metabolites in the urine agreed completely with that in earlier studies in healthy non pregnant subjects (6, 8). This result is somewhat puzzling in view of the raised activity of DAO during pregnancy which has been shown to alter the ratio ImAA/(MeHi+MeImAA) in the blood. It was greater than unity in pregnancy and less than unity in the non pregnant controls (13). The explanation for this discrepancy is probably that normally a very small fraction of the total amount of histamine released in the body is released directly into the blood stream. This interpretation is consistent with the very low urinary excretion of histamine usually found in normal pregnancy. The very high values of urinary histamine recorded by Bjuro et al. (11) in prolonged pregnancy and pre-eclamptic

fore exceptional. Two subjects excreted more than 1000 µg histamine one day. The highest histamine value found in the present investigation 220 µg (Mk) was recorded in a severe case of toxemia in which the fetus died while the 1000 µg values of Bjurö et al (1) were seen in moderate forms of toxemia. Other patients in both studies excreted normal amounts of histamine and it was not possible to identify a definite correlation to any clinical or laboratory findings. Bjurö et al also observed high histamine values several days after parturition but this finding was more common after a normal pregnancy. These two studies differ as regards the methods used for histamine determination and the use of the standardized diet and may also differ as regards the selection of the patients and their treatment. However, there is no obvious explanation for the disparity between these studies.

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A COMPARATIVE STUDY OF UTERINE ACTIVITY IN LABOUR INDUCED WITH PROSTAGLANDIN F_{2α} OR OXYTOCIN AND IN SPONTANEOUS LABOUR

II Characteristics of Uterine Activity and their Effect on the Progress of Labour

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fact In 76 women with spontaneous labour or labour induced with oxytocin or PGF_{2α} intra uterine pressure recorded during the first stage of labour A statistical analysis was performed of the intensity and frequency of contractions the total uterine activity and the ability of peak to-peak intervals and amplitudes of contractions The duration of the latent phase was shortened by 50% in induced labour compared with spontaneous while the active phase was less affected neither the intensity and frequency of contractions and uterine activity in Montevideo Units were of the same magnitude in spontaneous as in induced labour except from two cases of hyperactivity found in the induction groups The variability of intervals between contractions and amplitudes of contractions were also of the same degree in all groups and no overall tendency towards greater regularity was seen during progress of labour If irregular intervals between contractions were found when active cervical dilatation had begun the progress of labour was slower and more uterine work was needed for dilatation than when the uterine activity was more regular

During the last twenty years oxytocin has been the most widely used agent for the pharmacological induction of labour More recently prostaglandins have been tried for this purpose in a large number of clinical trials (2 4 21 25) It has been claimed by earlier investigators that labour induced by oxytocin or prostaglandins is in all respects indistinguishable from spontaneous labour (1 20 26) Oxytocin induced uterine activity has been reported to be more regular than that found in spontaneous labour (70) It is often stated that prostaglandins tend to provoke uterine hypertonus and incoordination even at moderate dose levels (22

23 25 26) In high doses or in patients unusually sensitive to the drugs it is well known that both oxytocin and prostaglandins may cause uterine hyperactivity which could prove dangerous to the fetus and sometimes even to the mother (11 14 20 22 28)

The duration of the first stage is on an average significantly shorter in induced labours than in spontaneous ones (10 13 24) This could have several explanations but possibly induction of labour even at optimal dose levels gives rise to uterine activity which is greater than that found under normal physiological conditions According to those who oppose the liberal use of induction agents they could cause ill effects in the fetus resulting in residual neurological damage (18 19 27)

To our knowledge no reports on systematic attempts to compare the characteristics of the uterine activity provoked by prostaglandins with other forms of labour have been published The purpose of the present study was to provide a basis for such a comparison and also to relate the different characteristics of the uterine activity to the clinical course of labour

MATERIALS AND METHODS

The patients studied were 76 women in labour Twenty four women were studied during spontaneous labour (Group N) 76 during labour induced with oxytocin (Group O) and 6 during labour induced with prostaglandin F_{2α} (Group P) The dose levels used were 2-16 mU/min for oxytocin and 3-15 µg/min for prostaglandin F_{2α}

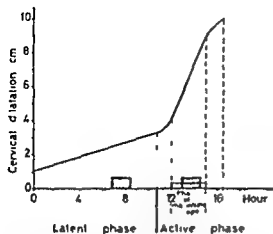


Fig 1 Partogram curve with nomenclature according to Friedman (13). Hatched areas indicate periods during which the cardiotocographic recordings were studied in detail.

All infants were liveborn, occiput—anterior vertex presentations. Relevant clinical details for the three groups of patients are presented in Table I.

Prior to induction the Bishop score (3) was assessed in all patients. During the course of labour vaginal examinations were made at approximately hourly intervals and the progress of labour was plotted on partograms according to Friedman (13). The patients with spontaneous labours were followed from the point of a cervical dilatation of 4 cm or earlier. In the induction patients the whole course of labour was studied.

The intra uterine pressure was recorded on cardiocardiographs from Hewlett Packard and Corometrics by open polyethylene catheters introduced transcervically. If intrauterine rupture of the membranes had not occurred amniotomy was performed at a cervical dilatation of 4–5 cm.

From the individual pressure recordings two sections (Fig 1) were selected to allow a detailed analysis of

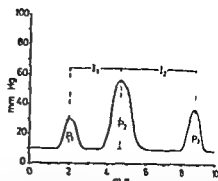


Fig 2 Schematic drawing of pressure recording showing intervals (I) and amplitudes (P) of contractions in mm Hg. Frequency (C) = 1/10 min. Uterine activity (P₁–P₃) MU.

20 successive contractions in each. The first was corresponded to the latter part of the latent phase of Friedman's cervical dilatation curve while the second corresponded to the phase of maximal speed of cervical dilatation in late active phase. Due to technical problems it was not possible to obtain satisfactory recordings from all patients, respectively in the O and P groups during the latent phase and from one patient in the O group during the active phase.

Both groups O and P had one patient each in whom uterine hyperactivity appeared in the end of the third stage. This was treated with orciprenaline. In these cases the values for the uterine activity before these episodes were included in the results.

The following characteristics of the uterine contractions were studied in a total of 115 observation periods (also Fig 2).

The intensity (P) of the contractions measured in mm Hg from the level recorded between the contractions to the peak of the contraction.

The frequency (F) of the contractions per 10 minutes.

The uterine activity in Montevideo Units (MU) (4).

The interval between the contractions (I) taken as

Table I Clinical data for the three groups of patients

Values given as means \pm 1 S.D. PP=Primiparae MP=Multiparae N=Spontaneous labour O=Induction with oxytocin P=Induction with PGF₂

	Group N		Group O		Group P	
	PP	MP	PP	MP	PP	MP
N	12	17	12	14	17	14
Week of pregnancy	40.9 \pm 1.4	40.6 \pm 1.0	41.9 \pm 1.1	41.3 \pm 1.4	40.6 \pm 1.7	39.1 \pm 1.4
Bishop score			7.0 \pm 1.7	6.8 \pm 1.0	4.8 \pm 1.5	4.5 \pm 1.1
Birth weight (g)	3.657 \pm 371	3.663 \pm 367	3.587 \pm 731	3.756 \pm 193	3.755 \pm 4.4	3.573 \pm 1.7
Infusion time (min)			503 \pm 716	273 \pm 174	503 \pm 153	411 \pm 1.1
Stage I (hours)	11.4 \pm 5.1	7.1 \pm 3.8	7.7 \pm 5.5	3.4 \pm 2.2	6.4 \pm 3.3	7.1 \pm 1.1
Stage II (min)	60 \pm 39	15 \pm 11	43 \pm 20	12 \pm 16	37 \pm 31	
Time for cervical dilatation 4–10 cm (min)	193 \pm 145	105 \pm 47	185 \pm 127	85 \pm 58	131 \pm 65	6 \pm 1
Maximum speed of cervical dilatation cm/hour	4.8 \pm 2.9	7.0 \pm 3.6	5.2 \pm 4.4	7.6 \pm 3.4	3.8 \pm 2.2	10.8 \pm 1.1

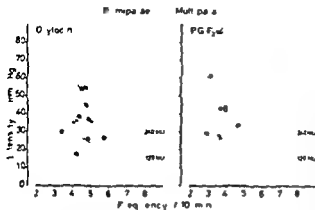


Fig 4 Individual values of mean frequencies and intensities of contractions during the latent phase of labour

tocin inductions in both primiparae and multiparae. The differences in this respect between the two drugs were negligible. The cervical dilatation curves for all groups showed a remarkable conformity to those given by Friedman. As expected, progress was much faster in multiparae than in primiparae.

The main difference in the rate of progress was a shortening of the latent phase in all the groups with induced labour. The maximum speed of cervical dilatation was less influenced by induction. The course of labour was very similar in the two groups in spite of the fact that the patients in Group P had significantly lower pelvic scores and less initial cervical dilatation than those in Group O.

The labour characteristics studied during the observation periods for the three groups of patients are listed in Table II.

In general, the frequency and intensity of the contractions increased slightly from the latent to

the active phase with a resulting increase in uterine activity measured in Montevideo units. Some significant differences were found in mean values between the groups of patients between primiparae and multiparae with respect to frequency but no consistent pattern could be revealed. No significant differences were found in mean intensities or uterine activities during the same observation periods.

The individual mean values for intensity and frequency of the contractions are plotted in Figs 4 and 5. The dashed lines in the figures refer to uterine activities of 120 MU and 740 MU, which are within the limits often given for uterine activity during active labour (6, 8). In spite of the fact that the different groups did not contain any cases of clinical dystocia, the individual values were large but of about the same magnitude in all groups. In the active phase, the values in the oxytocin group possibly showed somewhat less variation than values in the other P groups. A tachysystolic activity was seen in one mother in Group P during the latent phase. Apart from this case and two cases of uterine hyperactivity referred to under "Maternal complications", the individual values for intensity or frequency in the patients did not exceed those found in spontaneous labour. It is also evident from the figures that although the individual variations were wide, most of the values fall within the somewhat given normal limits. For the pooled values in the diagrams, a negative correlation was found between the intensity and the frequency of the contractions during the active phase of labour ($r = -0.4$, $P < 0.01$). This is in accordance with the slope of the theoretical lines for constant uterine activity in Figs 4 and 5.

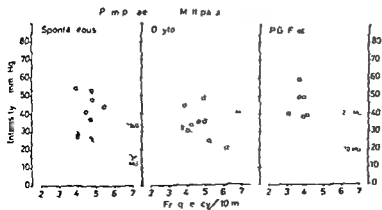
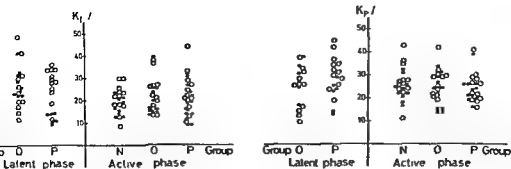


Fig 5 Individual values of mean frequencies and intensities of contractions during the active phase of labour



6 Individual values of the interval variability index (K_I) and the intensity variability index (K_P) in the groups

N O and P during the latent and active phase of labour
O Primiparae ● multiparae

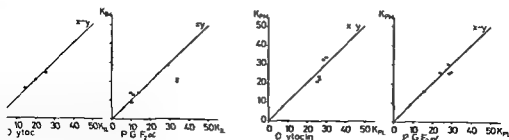
that the intensity of the contractions would have a dominant effect on the magnitude of the values is indicated by the distribution of the values in Figs 4 and 5. Accordingly the uterine activity in MU during active labour was more closely correlated to the intensity of contractions (0.82) than to the frequency ($r=0.31$).

Table II shows that the uterine work needed to obtain cervical dilatation was as expected much lower for primiparae than for multiparae. There were large individual variations within the groups. Differences in this respect between the three groups were generally fairly small, apart from the finding that prostaglandin induced multiparae had lower values than the multiparae in the O and N groups ($P<0.05$).

The variability of the intervals and the amplitudes within each recording was assessed by the indices K_I and K_P as described under Methods. The values of these indices varied between 8%–45% in the total series (Fig. 6). Significant differences were not found between the groups N and P. The index values showed no consistent

tendency towards lower values in multiparae than in primiparae but significantly lower mean K_I and K_P values were noted for the multiparae in Group P during the latent phase. From the first to the second observation period there was no general tendency towards a more regular activity (Fig. 7) although rather large changes in this direction occurred in some cases.

The degree of irregularity in the uterine activity assessed by the K_I and K_P was not found to correlate to the length of pregnancy or to pelvic score. Neither the intensity nor the frequency of the contractions correlated significantly with the interval variability index K_I in multiparae but not in primiparae; a weak negative correlation was observed between K_I and the mean uterine activity in MU ($r=-0.37$, $P<0.05$) which indicates that a high uterine activity in these patients was associated with more regular contractions. The intensity variability index K_P was found to correlate negatively with the mean uterine activity in MU found during the period (latent phase $r=-0.59$, $P<0.001$; active phase $r=-0.29$, $P<0.05$). In multi-



7 Changes in the variability of intervals (K_I) and intensities (K_P) of contractions from the latent (L) to active phase (A) of labour

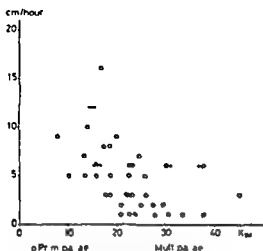


Fig 8 The maximum speed of cervical dilatation in cm/hour in relation to the interval variability index λ_I during the same period

parae the λ_I and λ_F were positively correlated during the active phase of labour ($r=0.43$, $P<0.01$).

Effect of the different variables of the uterine activity on the progress of labour. No clear relationship could be demonstrated between the frequency and intensity of the contractions or the activity in MU and the maximum speed of cervical dilatation. Of all the variables studied λ_I (Fig 8) was found to have the best correlation to the rate of cervical dilatation during the active phase of labour. The difference in cervical dilatation rate between patients with $\lambda_I > 20\%$ and $\lambda_I \leq 20\%$ respectively was highly significant when tested with Fischer's exact test of 2×2 tables ($P<0.001$). This was true for both primiparae and multiparae.

To test this relationship further the same plot as in Fig 8 was made for all patients with average frequency of 4–5 contractions/10 min. This revealed the same correlation between λ_I and labour progress. Similar correlations could be demonstrated in the induction cases for λ_I and the total length of stage I but not for λ_I and the length of the latent phase. No corresponding correlation between λ_F and labour progress was found during either the latent or active phase.

The degree of irregularity in the uterine activity also had a marked influence on the amount of uterine work necessary to achieve cervical dilatation during the active phase of labour. Figure 9 shows that in patients with high λ_I values more uterine work was needed to achieve one cm of

cervical dilatation. When the differences were tested with Student's *t* test $p<0.05$ was found in groups except for multiparae with induced labour.

DISCUSSION

As previously pointed out by Friedman (11), there is a significant difference in the clinical course between induced and spontaneous labour. In our study, in that of Friedman, the average duration of total labour was about 50% shorter in both primiparae and multiparae. It is evident from Fig 3 that this was mainly due to a shortening of the latent phase.

Considering that the length of the latent phase is thought to depend mainly on the time needed to soften and efface the cervix (13) it is remarkable that our prostaglandin inductions, in spite of significantly lower pelvic scores, had latent phases of the same duration as the oxytocin inductions. This is in agreement with our clinical impression that in patients induced by PGF $_2$ a rapid and marked softening of the cervix was noticed during the first part of the infusion without any other signs of labour progress. This phenomenon was not observed with oxytocin. It gives some support to previous statements that prostaglandins are effective induction agents in cases with an unripe cervix (2, 5, 26).

An obvious explanation to the short latent phase in the induction cases would be differences in uterine activity during this part of labour. Spontaneous labour often starts with weak, irregular contractions which gradually become stronger and more regular. On the other hand,

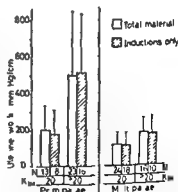


Fig 9 Uterine work (means and SD) needed per cm of cervical dilatation during the active phase of labour in patients with high and low values for λ_I .

successful inductions the doses of the oxytocic are adjusted to provoke regular and effective contractions more rapidly. This would tend to relatively short latent phases in spite of the fact that the cervical resistance probably is greater in many cases of induction.

Once labour was established, remarkably small differences were found between the groups N O, P and between primiparae and multiparae regarding the labour characteristics frequency, intensity or uterine activity. Thus induced labour could not be distinguished from spontaneous in this respect.

During both latent and active phase of labour the uterine activity stayed with few exceptions between 10 and 240 MU which is in good agreement with previous studies of the uterine activity during active spontaneous labour (6-8). This tendency explains the observed negative relationship between intensity and frequency during labour. This has been observed previously with regard to changes in frequency and intensity in the same individual (7, 20).

Lindgren (17) found in a series of spontaneous labours that for different levels of uterine activity expressed in MU it is possible to define an ideal frequency which gives the highest rate of cervical dilatation. Such an ideal frequency could not be demonstrated in our series. One reason for this discrepancy might be that Lindgren calculated the rate of cervical dilatation during the whole first stage while we studied the latent and the active phases separately. It is also possible that the oxytocic drugs given to most of our patients might have influenced the relationship between frequency and intensity (23). The values shown in Fig. 3, however, do not support this hypothesis. Furthermore the fetal weight and the circumference of the fetal head varied probably more in our series than in the study referred to above.

The amount of uterine work needed during the active phase of labour for each cm of cervical dilatation showed marked differences between primiparae and multiparae. Remarkably low figures for this variable were found in multiparae induced with prostaglandins. As intensities, frequencies and uterine activities were very similar in all groups, this finding confirms the generally accepted view that the average cervical resistance is much less in multiparae. The importance of the cervical resistance for the progress of labour is further

stressed by the fact that when effective labour had been established, no significant correlation could be demonstrated between frequency, intensity or the total uterine activity and rate of labour progress in any of the groups.

Once the uterine activity had reached the values referred to above, irregular intervals between contractions were found to have a marked negative effect on the rate of cervical dilatation during the active phase. High interval variability indices (Λ_1) were associated with significantly lower rates of cervical dilatation, longer duration of the whole first stage of labour and a higher amount of uterine work needed per cm of cervical dilatation, both in spontaneous and induced labours. Variations in intensity did not seem to have this clinical significance. It must be stressed that no significant or even suggestive differences were observed in the interval or intensity variability between patients with spontaneous labour and labour induced by oxytocin or PGF.

Diverging statements about changes in regularity of the uterine activity during labour have been put forward. Krapohl (15) in a small series of multiparae found a tendency towards greater regularity with increasing cervical dilatation. This was denied in another paper (9) from the same group. In our study of induced patients a clear tendency towards greater regularity during labour was found only in a few cases.

The results of the present investigation indicate that labour induced by oxytocin or PGF_{2α} in moderate doses cannot be distinguished from spontaneous labour. It must, however, be pointed out that all patients in this series were monitored by continuous tocographic recordings and that the rate of administration of the drugs was carefully adjusted to the degree of response in terms of uterine activity. As signs of overstimulation could rapidly be detected and the dose reduced or other types of treatment instituted, the results here presented are not necessarily valid for other dose schedules or in circumstances where the drugs are administered without tocographic control.

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BASIC LEVELS OF THE IMMUNO SUPPRESSIVE SERUM PROTEIN PZ IN WOMEN—INFLUENCE OF AGE PARITY GONADOTROPHINS AND ESTROGENS

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act The basic levels of the pregnancy zone protein were measured by a radio-immunoassay in 270 women of various age. All women were apparently healthy; no drugs. Values were correlated to age, parity, lotrophins and estrogens. A significant age dependent increase was found for PZ while no influence could be attributed to the other factors studied. The present study emphasizes that the age dependent variation of PZ concentration should be considered when clinical data of protein are evaluated.

pregnancy zone protein (PZ) is a human serum glycoprotein (1, 3, 15, 17, 20) with immunosuppressive properties in vitro (5, 14, 19). The serum concentration of PZ is known to increase during pregnancy and estrogen treatment with individual values around 500–1000 $\mu\text{g/ml}$ (3, 6, 7, 16, 22). Previously the assay of PZ has been based on electrophoresis or immunodiffusion techniques with a detection limit of 10–40 $\mu\text{g/ml}$ (3, 16, 22). A recently developed radio-immunoassay for PZ with a sensitivity of 32.3 ng/ml has made it possible to study the basic levels in non pregnant healthy individuals and values around 8 $\mu\text{g/ml}$ have been recorded.

The biological significance of this immunosuppressive serum factor is not clear. During pregnancy there is a depression of the maternal cellular immune response (10, 12). Physiological concentrations of PZ inhibit the reactivity of human lymphocytes (5, 14), a mechanism of possible importance for the immunological adjustment to pregnancy. Apart from pregnancy and estrogen treatment, moderately increased levels of PZ (50–200

$\mu\text{g/ml}$) have been reported in patients with malignant tumors and other diseases (2, 4, 18, 21). However, these reports should be evaluated with caution and the serum concentration of PZ in pathological conditions should be compared to the concentration in healthy individuals of corresponding age. At present no such studies have been reported. The individual variation in the PZ concentration during pregnancy and hormonal treatment is very marked and conclusions concerning the biological significance of PZ in various diseases entirely de-

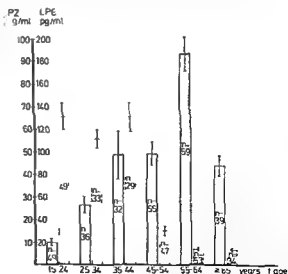


Fig 1 Distribution of mean values \pm S.E.M. for PZ $\mu\text{g/ml}$ (—) and low polar estrogens LPE pg/ml () in the total series according to age groups. Number of investigated individuals is indicated.

Table I Correlation within age groups between the serum concentration of PZ and LPE in 745 women

Age group	n	Mean \pm S.D.		Correlation coefficient (ρ)	$y = bx + a$	(b)
		PZ ($\mu\text{g/ml}$)	LPE (pg/ml)			
15-24	49	9.9 \pm 10.6	131.3 \pm 78.8	+0.33	$a = 4.2$ $b = 0.0$	n.s.
25-34	33	23.2 \pm 22.9	111.7 \pm 43.2	-0.20	$a = 34.9$ $b = 0.1$	n.s.
35-44	29	48.7 \pm 57.8	130.0 \pm 69.1	-0.26	$a = 77.6$ $b = -0.7$	n.s.
45-54	47	48.8 \pm 71.8	29.9 \pm 28.9	+0.04	$a = 49.3$ $b = 0.1$	n.s.
55-64	53	93.1 \pm 57.1	13.7 \pm 10.7	+0.11	$a = 83.8$ $b = 0.7$	n.s.
≥ 65	34	43.4 \pm 26.3	13.3 \pm 7.5	-0.12	$a = 50.9$ $b = -0.4$	n.s.

pend on further knowledge of the basic levels in healthy individuals. Furthermore the possible variation with age, endogenous estrogen concentration and previous pregnancies should be considered.

The present communication describes the serum levels of PZ measured by radio-immunoassay in healthy non pregnant women of different age and parity. The PZ concentration has been related to age, parity and serum levels of LH, FSH and estrogens.

MATERIALS AND METHODS

Blood samples were drawn from a total of 270 women of various age. After coagulation all sera were decanted and stored at -20°C until use. The samples were coded and tested blindly in duplicate. Samples were drawn from women attending different clinics at the University hospital Umeå or Sabbatsberg hospital Stock-

holm for health certificates for employment or general health control. All women were apparently taking no drugs. Data concerning age and parity were recorded. Information of parity was available only.

The serum concentration of PZ ($\mu\text{g/ml}$) was determined by a recently developed radio-immunoassay. Results for PZ are expressed as ng of the biologically active fraction (13-15) per ml.

In postmenopausal women, age ≥ 55 years, the determination of serum levels of FSH ($n = 84$) and LH ($n = 84$) was carried out by radio-immunoassays using the Double Body Solid Phase (DASP[®], Organon Oss Hofstra) radioimmunoassay procedure. Antibodies against FSH and LH were highly purified FSH and LH for iodination obtained from Kabir AB, Stockholm, Sweden. Results were expressed as mIU of Human Pituitary FSH and Human Pituitary LH or ICSH 68/40 per ml, respectively.

Low polar estrogens (estrone + estradiol)

Table II Correlation within age groups between the serum concentration of PZ and parity in 113 women

Age group	n	Mean \pm S.D.		Correlation coefficient (ρ)	$y = bx + a$	Significance of the (b)
		PZ ($\mu\text{g/ml}$)	Range parity			
25-34	26	21.7 \pm 18.0	0-1-2-7	+0.04	$a = 21$ $b = 0.6$	n.s.
35-44	24	35.6 \pm 21.7	0-1-2-7	+0.09	$a = 32$ $b = 1.8$	n.s.
45-54	28	54.6 \pm 76.6	0-1-2-7	-0.29	$a = 82.7$ $b = -11.9$	n.s.
55-64	35	97.1 \pm 58.4	0-1-7	-0.24	$a = 118.4$ $b = -9.8$	n.s.

II Correlation within parity groups between the serum concentration of PZ and age (15-60 years) in healthy women

n	Mean \pm S D PZ (μ g/ml)	Mean \pm S D Age	Correlation coefficient (ρ)	$y = bx + a$	Significance of the slope (b)
70	25.3 \pm 49.1	27.8 \pm 12.2	+0.64	$a = -47.0$ $b = 2.6$	$P < 0.001$
19	42.3 \pm 35.3	33.3 \pm 12.3	+0.59	$a = -24.2$ $b = 1.7$	$P < 0.01$
33	47.8 \pm 47.6	46.0 \pm 10.4	+0.45	$a = -46.4$ $b = 2.0$	$P < 0.01$
22	66.8 \pm 66.0	51.1 \pm 7.4	+0.43	$a = -128.4$ $b = 3.8$	$P < 0.05$

no-reactive estrogens") in serum were determined of the women by the radio-immunoassay technique (vist & Johansson (9). The antibody used reacts to with estradiol 17 β and to 50% with estrone (11). The values were expressed as pg immuno-reactive of equivalents per ml. The relation between different variables in statistical was calculated as product moment correlation (ρ). Tests of significance for co-variation were performed. The slope of the calculated straight line ($y = bx + a$) in the age groups values for PZ were compatible with assumption of a normal distribution.

RESULTS

The mean values for serum levels of PZ and low estrogens (LPE) in different age groups of the series are shown in Table I and Fig. 1. The results indicate an age dependent increase in the PZ level with the lowest mean value (9.9 \pm 1.5 μ g/ml) found for young women (15-24 years) and the highest mean value (93.1 \pm 7.4 μ g/ml) for women \geq 65 years of age ($p < 0.001$). Thereafter a decrease in PZ levels could be observed ($p < 0.001$). Within the age groups no correlation was found between levels of PZ and endogenous estrogens (Ta-

ble II). In order to study the influence of parity on the increase of PZ with age the correlation between PZ concentration and parity was calculated for different age groups. Women in the age groups 15-24 and \geq 65 years were not included for statistical reasons. Within the age groups no significant correlation between PZ level and parity could be found (Table II).

Fig. 1 indicates a decrease in the serum concentration of PZ in elderly women. Therefore when the correlation between PZ level and age was calculated, women over 60 years of age were excluded. The patients were grouped according to parity (0, 1, 2, 3). The correlation within the parity groups between the serum concentration of PZ and age is shown in Table III. A significant increase till the age of 60 was found.

The influence of age on the serum level of PZ is further illustrated in Fig. 2 which shows the mean values \pm S D for women between 17 and 77 years of age. The PZ value for each year is calculated as the mean from observations taken from women in an age group covering the given age \pm 3 years.

The serum levels of LH and FSH were determined in the postmenopausal women (>55 years).

IV Correlation between the serum concentration of PZ and FSH in healthy women \geq 55 years of age

n	Mean \pm S D PZ (μ g/ml)	Mean \pm S D FSH (mIU/ml)	Correlation coefficient (ρ)	$y = bx + a$	Significance of the slope (b)
53	96.6 \pm 58.0	67.2 \pm 77.33	-0.1	$a = 113.0$ $b = -0.3$	n.s.
31	45.4 \pm 77.5	51.4 \pm 78.44	+0.04	$a = 43.3$ $b = 0.0$	n.s.

Table V Correlation between the serum concentration of PZ and LH in healthy women ≥ 55

Age group	n	Mean \pm S D PZ ($\mu\text{g/ml}$)	Mean \pm S D LH (mIU/ml)	Correlation coefficient (ρ)	$y = bx + a$	Significance of the slope (b)
55-64	51	97.7 \pm 58.8	69.9 \pm 20.3	0.14	$a = 70.1$ $b = 0.4$	n.s.
≥ 65	30	44.7 \pm 27.2	55.2 \pm 10.2	-0.05	$a = 48.3$ $b = -0.1$	n.s.

No significant correlation between levels of PZ and the gonadotrophins could be found (Tables IV and V).

DISCUSSION

The concentration of the immuno-suppressive serum factor PZ showed pronounced variations with age. Levels were rising from the age of 15 up to around 60 years. This increase was independent of parity and endogenous estrogen concentration. In elderly women, approximately after the age of 60 years, there was a decrease in the PZ concentration. A tenfold increase with age in the basic levels of PZ was found when young women were compared with older ones.

As in previous investigations (6, 16) the variation between individuals was considerable (Fig. 2). Thus PZ concentrations of 200 $\mu\text{g/ml}$ were found within 2 S D from the mean value between 50-65 years of age. Values of this magnitude have previously been attributed to various pathological conditions (2, 4, 18, 21). The present results emphasize

that the age dependent variation in PZ concentration should be considered when such data are used.

Estrogen is believed to be important in the induction of PZ and the increase of this serum during pregnancy and hormonal treatment is well established. After delivery the high serum PZ in pregnancy are reduced to non-pregnant within six weeks (16). Furthermore a decrease in PZ concentration is found after a month of estrogen treatment (6). A positive correlation between serum levels of LH and PZ could therefore be expected. If such correlation was found in any of the studied age groups (Table I) the role of LH as the direct trigger in the induction of PZ could therefore be modified. Estrogens must be regarded as inducing factors for the synthesis of PZ but there might exist some kind of physiological threshold level for the action of estrogen. Also an interaction between estrogen and other hormones must be considered. The

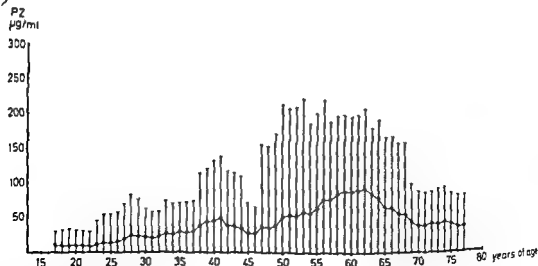


Fig. 2 The mean concentration \pm 2 S D of PZ $\mu\text{g/ml}$ at various age in healthy women between 17 and 77 years. The PZ value for each year is calculated as the mean from observations taken from women in age groups the given age ± 3 years.

levels of estrogens and the increasing levels of women around 50 years of age indicate the presence of other inducing agents for the synthesis.

An influence of gonadotrophins in this respect seems unlikely as there was no correlation between levels of FSH/LH and PZ in postmenopausal women (Tables 4 and 5). The nature of the factors responsible for the induction of PZ synthesis is at present unknown.

The immuno-suppressive properties of this serum should have biological relevance in the physiological adaption to the fetal allograft during pregnancy. The mechanisms for its induction and regulation are still unknown.

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DAKTAR resorberas i mycket ringa grad vid lokal applikation.

DAKTAR har goda kosmetiska egenskaper och innehåller ej lanolin eller parabener. Ett fall av sensibilisering har rapporterats. Krämen färgar ej kläderna och kan avtvättas med tvål och vatten.

Klinik: DAKTAR är effektivt vid vaginiters orsakade av Candida spp. och andra svampar.

ter. Såväl mykologisk som klinisk utläkning har konstaterats i ca 90% av de behandlade fallen. Vid behandling med DAKTAR erhålles en snabbt insättande effekt med snabb lindring av symtom som sveda, klåda och flutor. Behandling med DAKTAR sänker förhöjda vaginala pH-värden vilket har en gynnsam effekt på den naturliga bakteriefloras tillväxt. DAKTAR lämpar sig väl för behandling av symptomlös vaginal mykosa hos gravida som profylax mot oral mykosa hos det nyfödda barnet.

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Förpackningar: Tub à 78 g med applikator.



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OCCURRENCE OF GENITAL HERPES SIMPLEX AND CYTOMEGALOVIRUS INFECTIONS IN PREGNANCY

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Eero Saksela and Pauli Leinikki

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A total of 244 pregnant women 172 in the first and 72 in the third trimester were screened for genital herpes simplex (HSV) and cytomegalovirus (CMV) infection using virus isolation cytological examination and serological studies. In addition immunofluorescence staining of exfoliated cervical cells was used for the detection of HSV infections. Herpes simplex virus (HSV) was not isolated and no characteristic HSV altered cells were found in cytological (PAPA) smears. Cytomegalovirus was isolated four times but PAPA smears obtained from these patients were also normal. In 34 patients HSV antiserum detected immunofluorescence positive exfoliated cervical cells. Overall 14% of patients had HSV 2 antibodies and 71% CMV antibodies. No differences between the first or third trimester in these respects were noted.

Herpes simplex cervicitis during labour is a serious infection for the newborn infant (16). Because the risk can be diminished by caesarean section (1) accurate and rapid diagnosis of this clinically often inapparent infection is important. In contrast to herpes simplex virus (HSV) infection cytomegalovirus (CMV) infection during delivery does not seem to be dangerous to the newborn child (14) although intrauterine infection may cause serious damage to the fetus (3).

The available methods suitable for routine diagnosis of herpetic infections are virus isolation serological studies observation of cytological alterations in cervical smear preparations (PAPA) and immunofluorescent staining of exfoliated cervical cells for detection of viral antigens. Timonen et al (11) were not able to find herpes simplex virus infections among 175 asymptomatic

pregnant women. Jeansson and Molin (4) reported an 0.5% incidence of HSV infection among asymptomatic females. Roughly 10% of asymptomatic pregnant women have been found to exfoliate HSV antigen containing cervical cells detectable by fluorescent antibody (FA) technique (16, 25). Variable incidences up to 28% during the third trimester (14) have been reported for clinically inapparent cytomegalovirus infections among pregnant women.

Cytological diagnosis of gynecological herpes simplex infections has shown approximately 70% accuracy as compared with virus isolation (13). On the other hand in a prospective study in a venereology clinic (10) cytology has proved to be even more accurate than virus isolation in preclinical cases of infection whereas in advanced clinical stages of infection with necrotizing lesions cytology may be less reliable. In contrast to herpes simplex virus infections only few prospective studies exist about the value of cytology in the diagnosis of CMV infections (10, 2).

Immunofluorescent staining for the detection of HSV antigen has been used successfully for the diagnosis of herpes simplex infections in cases where ulcerative lesions are present (7) but reports do not exist of the value of the fluorescent antibody technique as a screening method.

A prospective screening study was undertaken to determine the occurrence of herpes simplex and cytomegalovirus infections in a group of pregnant women and to compare cytological virological and immunofluorescent methods in the diagnosis of these infections.

Table I Virological and cytological findings among 244 pregnant women

	Study group				Total group	
	1st trimester		3rd trimester			
	n	%	n	%	n	%
HSV excretors	0		0		0	
FA positives ^a	24	14	10	14	34	14
HSV 2 antibodies	24	14	11	14	35	14
No HSV antibodies	35	20	19	26	54	22
CMV excretors	3	2	1	1	4	2
CMV antibodies	123	72	50	80	173	71
Cells suggestive of HSV or CMV infection in PAPA smears	0		0		0	
Total	172		77		244	

^a 157 and 11 patients studied in the groups respectively

^b Immuno-fluorescent staining (FA) with HSV 2 rabbit hyperimmune sera and anti rabbit conjugate

MATERIALS AND METHODS

Patients The group consisted of 244 randomly selected pregnant women attending the outpatient clinics for legal abortion and maternity care of the I-II Departments of Gynecology and Obstetrics, Helsinki University Central Hospital. A total of 172 patients were in their first and 72 in their third trimester and all were less than 36 years old.

Cytology The cytological smears obtained from the posterior vaginal fornix, the ecto- and endocervix were spread across the same slide (74). The slides were fixed with propanol fixative and stained according to Papanicolaou's original method.

Immuno-fluorescent (FA) studies Cells for immuno-fluorescence were obtained with cotton swabs from the ecto- and endocervix and dispersed on slides. After air drying the slides were fixed with acetone at +4°C for four min. Immuno-fluorescent staining of fixed cells has been described earlier (7). Rabbit hyperimmune sera were used for HSV 1 and HSV 2. The sera were produced by infecting rabbits via the ocular route. The working dilutions were selected by titrating their antibody titer on infected continuous monkey kidney (BSC₁) cells. Four units of type 2 antiserum and 1-1.5 units of type 1 antiserum were used. In this dilution type 1 antibodies did not react with cells infected with type 2 HSV.

Commercially available FITC conjugated anti rabbit gamma globulin was used as described earlier (7). Five spots were stained on each slide: two with type 2 antiserum, two with type 1 antiserum and one with conjugate only.

Virus culture isolations Primary human embryonic fibroblasts (HES) were inoculated with samples taken with cotton swab from the cervical canal and the posterior fornix separately. The samples were immediately transported to the virological laboratory where the inoculation took place. The methods for identification of isolated viruses have been described earlier (6, 8).

Serological tests The methods for anti HSV and CMV antibody assays have been described earlier (5, 8).

RESULTS

Virus isolation

All patients were clinically asymptomatic. 244 patients, 220 could be successfully virus isolated. In the remaining 24 virus cultures were destroyed by microbial growth. The rate of successful isolations is presented in Table I. Herpes simplex (HSV) could not be isolated in any of the while four patients excreted demonstrable megalovirus (CMV). Three of the latter were from first trimester patients and one from trimester patient. Immuno fluorescent cytological smear preparations HSV antigen was positive in 24 first trimester and ten third trimester patients (Table I).

Serology

Serological studies showed that approximately same proportion of patients in early and late pregnancy had HSV antibodies and the same when antibody patterns compatible with activity were compared. Neither did the mean HSV titers in the two groups differ significantly. Of patients who exfoliated HSV positive cervical cells, 11 were antibody positive and four had type 2 antibodies which corresponded with the general distribution in the whole group.

Cytomegalovirus antibodies were demonstrated in 173 patients (71%). Among those excreting one had a moderate or high titer (over 800), a low titer (20-40) and one was seronegative. Neither could significant differences be

in the first and third trimester groups in the occurrence of cytomegalovirus antibodies. The serological data are presented in Table 1.

ogy.

Morphological signs of viral infection were seen in PAPA smears included in this study. Patients in whom CMV was isolated were invited to a visit and another smear was taken 2-3 weeks after the original samples. These were also normal although special attention was paid to obtaining abundant endocervical samples.

DISCUSSION

Diagnostic difficulties usually arise in patients with ulcerative genital lesions, but the problem lies in the high proportion of asymptomatic HSV 2 infections in pregnant women (12-22). In the present study no herpes simplex viruses could be isolated although any cytological preparation should show evidence of herpesvirus infections. Pannu & Sigel (15) were unable to recover viruses from 229 cervico-vaginal specimens and HSV isolations performed by Montgery et al. (9) in 176 pregnant women with no clinical evidence of HSV infection were also negative.

In the present study virological samples were collected both from the endocervix and the fornix to obtain reliable samples for culture. It has been shown that the characteristic cellular alterations provide a high degree of accuracy in the diagnosis of herpes infections (22, 23) and that cytology can be even more reliable in the diagnosis of precancerous cases of HSV infections than virus isolation (10). Since also PAPA smears were negative, the results indicate the infrequency of HSV in asymptomatic obstetrical patients. In a recent study by the present authors (22) of gynecological patients in our clinics an 0.16% overall frequency of genital HSV infection was detected among 57 117 PAPA smears.

CMV was isolated four times and all of them from clinically asymptomatic patients. PAPA smears were all normal and even in repeated PAPA smears signs of cellular alterations compatible with CMV infection could be seen. Although a high percentage of obstetrical patients, up to 28% in some series, are reported to excrete cytomegalovirus in typical altered cells have been reported only once in cervical PAPA smears (2, 10). The reasons for the difficulty in detecting CMV altered cells are

not clear since under *in vitro* conditions CMV readily infects and causes typical morphological alterations in endocervical epithelial cells (21).

Immuno-fluorescent staining of exfoliated cells for the presence of HSV antigen has been successfully used for the laboratory diagnosis of herpetic infection in venereal and other groups of patients (7-20). Royston and Aurelian (19) were able also to demonstrate positive staining of exfoliated cervical cells from patients with cervical carcinoma. In the present study, however, it is evident that when used for screening of asymptomatic patients the correlation between positive fluorescent antibody (FA) staining and virus isolation is clearly inferior to the correlation found in clinically manifest cases. FA positive patients in this study showed a distribution of HSV antibodies similar to the whole group: 32% of FA positives and 22% of the whole series were HSV seronegative. Royston and Aurelian (19) suggested that the physico-chemical conditions in exfoliated cells may give rise to an abortive viral cycle and formation of HSV specific proteins. We cannot either rule out the possibility that the positive FA staining is not related to HSV infection but rather reflects a cross reacting antigenic structure on the surface of exfoliated cervical cells.

The analysis of antibody patterns in our material revealed that the proportion of HSV 2 carriers was about the same as those obtained in other areas with socio-economically comparable populations (18). The frequency 14% is slightly lower than that in another study on the Finnish population (17). The frequency of CMV antibodies among pregnant women seems to vary according to e.g. socio-economic, ethnic and geographic factors (14, 9). In the present study 71% of the women had CMV antibodies.

In our study all the children born to mothers with positive FA for HSV or excreting CMV during pregnancy were followed through their neonatal period. None of them showed signs of intrauterine or neonatal HSV or CMV infection. The risk to the child caused by a clinically manifest herpes simplex infection during pregnancy has been evaluated to be about 50-60% and can be reduced by abdominal delivery (1). The number of HSV 2 seropositive mothers far exceeds the number of neonatal infection cases, indicating that only a small proportion of the asymptomatic HSV 2 carriers do transmit the virus to the baby.

Although 14% of patients in the present study

had HSV 2 antibodies and 71% CMV antibodies indicating a latent infection no morphological or virological evidence could be obtained of productive infection by herpes simplex virus and only four times could CMV be isolated. In the study by Nahmias et al (12) concerning cytologically detected genital herpes simplex infections during pregnancy in a low socio-economic group one per cent incidence was reported. This was about three times that found outside pregnancy and puerperium in the same population. Statistical analysis of the present results reveals that within 95% confidence limits the frequency of HSV infections in this population remains under 1.5%. However the quite low proportion of HSV 2 antibody positive patients in this group as well as the earlier retrospectively detected 0.16% frequency in our general outpatient population (22) would point to a lower incidence than that reported by Nahmias et al (12).

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SIMPLE HYSTERECTOMY IN THE PRESENCE OF INVASIVE CERVICAL CANCER

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Abstract. During the years 1956-69 74 cases of invasive cervical carcinoma treated by simple hysterectomy were studied at the Norwegian Radium Hospital. The 5 year survival for those with free operative borders was 77.1% compared with 30.7% for those cases in which the cervix was transected during operation. Before operation a supposed benign condition cancer should always be ruled out. If simple hysterectomy is performed and cervical cancer found in the specimen immediate postoperative megavoltage radiotherapy affords the best chance of

tions with special emphasis on the exclusion of cancer. Ideally this assessment must include adequate visualization of the vulva vagina and cervix cytological examination of the cervix the smear being taken from both the endocervical canal and the posterior fornix colposcopic examination and biopsy of any suspicious lesions and fractionated curettage if the transformation zone cannot be clearly seen or the patient has suspicious symptoms. Only after these investigations have been made can one be reasonably satisfied that the patient is ready for surgery.

This report deals with experience at the Norwegian Radium Hospital in managing the patient treated by simple hysterectomy for a presumed benign condition only to find carcinoma of the cervix in the operative specimen at histological examination.

CLINICAL MATERIAL AND MODE OF THERAPY

In the years 1956-69 74 patients were referred for further treatment to the Norwegian Radium Hospital after their initial operation had proved inadequate. Of these 48 have been observed for at least 10 years. During the same period 4492 patients with invasive cervical cancer received all their treatment in the hospital.

Table 1 lists the preoperative diagnoses. As many as 28 of these women presented with a cancer suspicious history of metrorrhagia. Another 3 were operated with a simple hysterectomy after a positive diagnosis of cancer had already been made at biopsy. Thus there are 31 in the series who had incompetent medical care. Many of those with a history of menorrhagia had been curetted several times before with negative findings. Two were operated upon for pelvic sepsis one with bleeding in pregnancy and one who presented with a subacute intestinal obstruction.

Only cases with proven histological evidence of invasion have been considered. All cases of microinvasion

accepted methods of treatment of invasive cervical cancer are either radical surgery radiotherapy or a combination of both. All workers in the field agree that simple hysterectomy is an inadequate form of treatment. Various authors have treated series treated inadequately in the first instance and offered suggestions for improvement in diagnostic accuracy and salvage. In 1968 Pearce & Brunschwig suggested that early radical surgery offered the most comprehensive therapy in the shortest time. In their opinion delay in the form of preoperative radiotherapy severely compromised the chances of cure. On the other hand Cosbie proposed radiotherapy as a reasonable alternative to radical surgery for salvage. Both, however, offered poor prognoses.

In 1973 Andras Fletcher & Rutledge presented a study of 148 cases in which they reported that in early stages of the disease the prognosis may be as good as in those treated more conventionally. Delayed radiotherapy was given immediately after hysterectomy. The most obvious way to reduce the error of patients inadequately treated is to improve the standard of preoperative assessment in all cases being operated for supposed benign condi-

Table I *Indications for hysterectomy*

Intermenstrual and/or post-coital bleeding	16	31
Post menopausal bleeding	3	
Bloody discharge	9	
Invasive cancer	3	
Carcinoma in situ	4	74
Myoma uteri	3	
Menorrhagia	11	
Others or unrecorded	25	
Total	74	

(stage Ia) are excluded. For ease of comparison we have divided the cases into groups (Table II) such that group I corresponds to clinical stage Ib, group II represents those with known gross disease cut through at operation and referred within 6 months of the original surgery whilst group III are those with tumour transected at operation but whose follow up treatment was delayed more than 6 months. In this scheme we have followed roughly the guide set by Andras *et al* (1).

There were 65 (87.8%) squamous cell carcinomas and the remaining 9 (12.2%) were adenocarcinomas, adeno-squamous or undifferentiated carcinomas.

60 (81%) were less than 60 years old whilst the average age was 49.9 (Table III).

Most patients were treated with intravaginal radium and external radiotherapy. Initially this external therapy was delivered by orthovoltage machines but after 1958 either a 31 MeV Betatron or a 60° machine was used. Table IV shows the methods of irradiation. Two patients had supravaginal hysterectomies and these received radium into the cervical stump in addition to intravaginal radium and external megavoltage therapy. During the early years of this study there was no set system of treating these patients and this is reflected in the spread of therapeutic regimes. The majority received radium and megavoltage therapy. In recent years standard treatment has been external megavoltage radiation delivering 5000 rad in the

Table III *Age distribution*

Age	Numbers
40-49	11
50-59	31
60-69	18
70-79	11
80+	3
Total	74

midpelvic plane. In the earlier years of the study orthovoltage machines were used, the dose was about 3000-4000 rads.

RESULTS

Tables V and VI show the status of the patients at 5 and 10 years. Of the 74 in the study 45 were free of disease at 5 years giving a survival of 60.8%.

Considering group I the 5 year survival was 77.1%. In this group there were 7 patients died of incurrent disease—one of a haemorrhage and the other of lymphatic leukaemia. Autopsy revealed no evidence of disease. A further 3 were alive after 5 years with active disease. None was considered for surgery, one received Thiotepa and Gamma therapy for an adenocarcinoma recurrence and the 3 she is the only one living now 9 months beginning this treatment.

In group II the 5 year survival was 37.5%. One patient had a transurethral resection for a recurrence after 2 years and is now alive with evidence of disease 12 years after therapy.

As could be expected the results in group III were not nearly so good—20% at 5 years and one was alive after 10 years.

Table VII records sites of recurrence of disease.

Table II *Findings at operation and time of referral*

Patient group		Observation time	
		5 years	10 years
I	Free operative borders (stage I)	48	29
II	Not free borders referred within 6 months	16	17
III	Not free borders referred after 6 months	10	7
Total		74	48

Table IV *Method of irradiation*

	Patient group	
	I	II
Vaginal radium	7	1
Radium + orthovoltage	6	1
Orthovoltage	—	8
Radium + megavoltage	24	4
Megavoltage	11	1
Others	—	—
Total	48	16

V Status at 5 years

Alive	Alive with disease	Dead of cancer	Dead of intercurrent disease	Lost to follow up
37 (77.1%)	3	4	2	2
8 (37.5%)	—	10	—	—
2 (10.0%)	—	8	—	—
45 (60.8%)	3	22	2	2

less of time interval after treatment Pelvic recurrence was by far the most common either or in combination with distant spread seen usually in the more advanced cases In this there were 13 whose cause of death was recorded as uraemia as a result of ureteric obstruction in the pelvis

Considering that these patients were mishandled initially the complications were surprisingly few two major ones were both found in group II first had a local resection of a bladder recurrence which eventually resulted in a chronically infected bladder requiring urinary diversion is alive and well with an ileal bladder 12 years after initial therapy The other had severe radiation damage to the bowel with multiple fistula formation She had several operations including colostomy and ureterostomy but died 2 years after surgery from chronic renal infection and electrolyte imbalance She had received radium and 3000 megavoltage

Table VII we see the fate of those with adenocarcinoma From this small series one cannot see difference in behaviour from those with squamous carcinoma but the overall figures are too small All those still living have survived for at least 5 years since the time of diagnosis Recurrences followed the common trend predominating in the pelvis

VI Status at 10 years

Alive	Dead of cancer	Dead of intercurrent disease
23 (79.0%)	4	2
4 (33.3%)	8	—
0 (0.0%)	7	—
27 (56.7%)	19	2

COMMENTS

Group I of the present series showed a relatively high 5 year survival rate As a matter of fact comparison with the survival figures for stage Ib lesions treated conventionally at the hospital during the same time period did not reveal any difference This reflects surely the selection of operative cases Only those who are relatively young and fit are candidates for surgery for benign conditions Furthermore the tumour was in most cases very small It should be emphasized however that all of them showed invasion more than 5 mm into the stroma

As expected the prognosis was poor in those cases where the tumour was transected during operation The poorest prognosis concerned group III where follow up treatment was delayed more than 6 months after simple hysterectomy The results presented here are in complete agreement with the series published by Andras et al (1)

From our series of only 9 cases of adenocarcinoma we cannot draw any firm conclusions but it would appear that the prognosis in adenocarcinoma is no worse than in the squamous disease

Table VII Sites of recurrence

Sites of recurrence	Patient groups			Total
	I	II	III	
Vagina and pelvis	1	—	1	2
Pelvis	4	4	2	10
Vagina and distant metastases	1	—	—	1
Pelvis and distant metastases	—	3	3	6
Distant metastases	1	2	2	5
No. of recurrences/Total No	7/48	9/16	8/10	24/74

The obvious conclusion of the present study is that it is still necessary to improve the standard of preoperative assessment in all women being operated for supposed benign conditions. All cases should be fully screened preoperatively using cytology, colposcopy, biopsy of any suspicious lesion and if indicated, fractionated curettage. If in spite of these measures an occasional case of invasive cancer is overlooked, immediate postoperative megavoltage radiotherapy should be given so as to ensure the best chance of cure.

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ULTRASONIC DIAGNOSIS OF PLACENTA PRAEVIA

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act 741 women suspected of placenta praevia were referred to ultrasonic scanning during the second and/or trimesters of pregnancy. In 61 an abnormal placental site was diagnosed. During Caesarean section carried out in 46 of these cases the location was compared with the ultrasound findings. The scanning diagnosis during the second trimester correlated with the findings at delivery. At delivery after the 35th week the diagnosis differed in only 10 cases. In all 4 there was a question of a low lying placenta versus partial placenta praevia. During the second trimester diagnoses of low lying placenta were repeated in several cases at later scans. It is discussed whether these early diagnoses of an abnormal placental site which later becomes normal indicate a migrating placenta and/or an uncertainty of the scanning method. Diagnosis of low lying placentae should be checked at repeat scans.

etermination of placental location has always been of importance in obstetrics, especially in patients with bleeding episodes during pregnancy or abnormal presentations. Recently increased use of diagnostic amniocentesis has created a need for accurate location of the placenta at all stages of pregnancy. Since 1966 (5, 6) ultrasound scanning has gained increasing ground in placental diagnosis, especially when the ultrasonic equipment had been improved sufficiently to visualize also placentae located on the anterior wall of the uterus (15). Comparative studies have shown results equivalent with other diagnostic methods, such as soft tissue X-rays (1) and isotope scanning (2, 3, 4, and 16). However, ultrasound scanning is preferred especially because of its better delineation of the fetal-placental border. Ultrasound scanning immediately prior to Caesarean section has given an almost 100% agreement with the operative findings in placental location (6, 15).

At ultrasound scanning at different stages of pregnancy there is an increasing discrepancy between the diagnosis and the results at term; the earlier the ultrasound scanning is performed. Thus Kukard (12) found a difference of 17.3% when scanning was done before the 30th week, 11% between the 30th and 34th week, and 4% between the 35th and 40th week. Despite the diagnostic uncertainty of ultrasound scanning it is still the only applicable method for locating the placenta during early pregnancy.

MATERIAL AND METHOD

The material comprises 741 patients scanned by ultrasound during the second and third trimesters of pregnancy for the purpose of locating the placenta. The indications were bleeding during pregnancy or irregular presentations. Of these patients 182 were examined during the second and 559 during the third trimester. Partial or total placenta praevia or low lying placenta (8.2%) were diagnosed in 61 patients. These 61 patients had a total of 145 scans: 46 during the second and 99 during the third trimester. Forty-six were delivered by Caesarean section.

The ultrasound scans of the placenta were carried out as routine examinations by the Ultrasonic Laboratory staff. Determination of the placental site during the Caesarean section was done by the changing members of the surgical staff.

The ultrasonic equipment was: ESKOLNE 20 rebuilt for B-scanning, Diasonograph NE 4107, and Diasonograph NE 4102 B.

RESULTS

Table I lists the ultrasound diagnoses and the findings at delivery in 63 patients. In the case of different scanning results the last one is indicated by an arrow.

Fourteen consistent investigations showed total



Fig 1 Longitudinal scan. Symphysis at left. Total placenta praevia (arrow). Ultrasonic scanning in the 37th week of gestation

centa praevia (Fig 1). In these cases all the ultrasound diagnoses were finally confirmed during the third trimester. In one case diagnosed by scanning as total placenta praevia, the diagnosis at delivery was partial placenta praevia. Two patients had a scanning diagnosis of total placenta praevia during the 14th and 17th week respectively. Both were re-scanned in the third trimester and at that time the location of the placenta was normal in both, but low on the lateral walls.

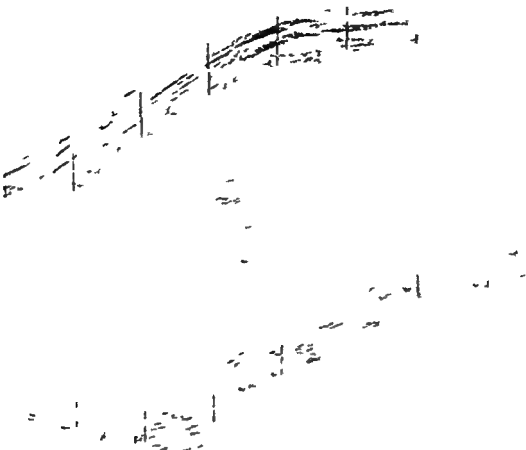
Five partial placenta praeviae were consistent. In four cases a placenta praevia diagnosed as partial at delivery had been deemed total during scanning and in two cases diagnosed at scanning as partial placenta praevia, operation showed low lying placenta. In seven cases partial placenta praevia had been diagnosed during the second trimester. Six of them were re-scanned during the third trimester and then showed a normal location. Of these

seven patients four had posterior wall placenta, two lateral wall placenta and one anterior wall placenta (Figs 2 and 3).

All 26 patients in whom ultrasound scanning indicated low lying placenta had been scanned in the third trimester. Of these 16 according to operative findings, two were found to have placenta praevia and two partial. The latter were posterior wall placenta. Six cases diagnosed at the first scanning as low lying placenta were at a normal site when re-scanned. All six patients with primarily abnormal findings had posterior or lateral wall placenta.

In two cases in which the ultrasound diagnosis was a normally situated placenta, Caesarean section revealed total and partial placenta, respectively. One was bipartite and the other placenta membranacea.

All the remaining 658 patients had a



Longitudinal scan. Symphysis at left. Partial placenta praevia (arrow). Ultrasonic scanning in the 24th week of gestation.

Table 1. Sixty-three patients in whom ultrasonic scanning or diagnosis at delivery showed abnormal position of the placenta.

Prenatal diagnosis				Diagnosis at labor			
Complete placenta praevia	Partial placenta praevia	Low lying placenta	Upper segment placenta	Complete placenta praevia	Partial placenta praevia	Low lying placenta	Upper segment placenta
				14	1		2
5			2		5		
4				4			
7			6			2	7
		16		2		16	
		6			2		
		6	6	1			6
		1	1		1		
			1				

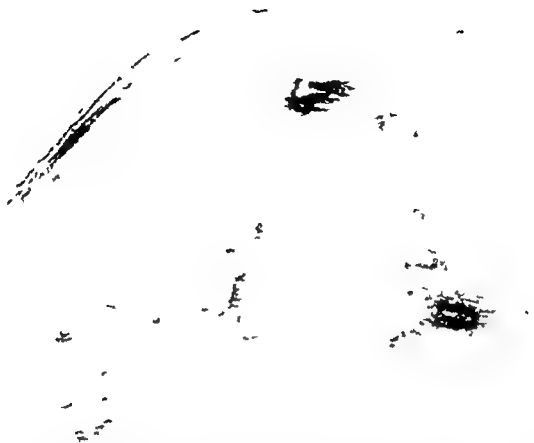


Fig 3 Longitudinal scan. Symphysis at left. Same patient as in Fig 2, now in the 34th week of gestation. Now the

placenta is normally situated on the anterior wall (arrow).

ated placentae at ultrasound scanning as well as partu

DISCUSSION

Analysis of the results suggests two factors in particular which require further assessment.

In the first place the agreement between ultrasonic diagnosis and assessment at delivery seems the worse the earlier the scanning has been performed.

The second problem is the difficulty involved in the exact visualisation of the lower margin of the placenta, especially on the posterior and lateral walls.

It is apparent firstly that placentae deemed at scanning during early pregnancy to be low lying are often found to be located the higher in the uterine cavity the closer to term the scanning is repeated. This is in accordance with the findings of

Hellmann (7). At ultrasound scanning during the first trimester he found only 1/10 of the implanted ova to be clinically placenta at term. An interpretation of such discrepancies being due to an erroneous first diagnosis is unjust, however.

In general the placenta is believed to be at a given site in the uterine cavity but several factors may account for an altered placental position. Around the 16th week the placenta covers the entire uterine cavity but at delivery only between one-third and one-quarter. Thus the original relationship between the placenta and the uterus changes in the course of pregnancy. The increase in uterine growth is not equal in the upper and lower segments. While the upper segment increases in mural thickness, the lower segment grows through stretching and distention of the fibres. This contributes to increasing the dis-

een the lower placental margin and the inter-
 uneven growth of the uterine layers also
 shifts in the placenta which according to the
 ry of King (10) must entail changes in its posi-
 and immediate repairs if an abruption is not to
 It
 or technical reasons an exact visualisation of
 lower margin of a low lying placenta is difficult
 4) especially during early pregnancy (12) This
 ies particularly to placentae situated on the
 erior and lateral walls as adequate filling of the
 der affords a more precise diagnosis in the case
 terior wall placentae. If the foetus is lying high
 ie uterus there is a tendency to under estimate
 distance to the os because the area is not dis-
 ed by the foetus. But if the head is well down in
 pelvis it may cover the impulses so that a
 edge of a posterior or lateral wall placenta is
 properly visualised. To elucidate these factors
 e is a possibility of using Donald's full bladder
 inique (5) or Sander's water pessary (13). By
 ns of a special pessary—filled with water and
 ed in the vaginal fornix—the poor visualisation
 anterior and lateral wall placentae may be im-
 ved.
 he appearances around the lower margin of the
 enta are more distinct close to term. In ques-
 able cases therefore a repeat scan should be
 mended as close to term as possible.
 i the present analysis there were no false posi-
 or false negative results after the 35th week but
 e were four cases of divergent interpretations
 cerning the diagnosis low lying placenta partial
 enta praevia or total placenta praevia (6.3%).
 The two false negative results that occurred were
 partite placenta in which only one part was
 alised and a placenta membranacea whose
 gin in the lower segment was not visualised.
 e were scanned before the 34th week.
 he other differences apparent from Table I do
 decisively reduce the value of the method. In
 evaluation regard must be paid to the fact that
 Caesarean section was carried out at different
 es of labour and by changing surgeons. Both
 e influenced the operative assessment regarding
 ental location.
 e must be concluded that in a number of cases
 placenta migrates towards the fundus in the
 se of pregnancy and that visualising the lower
 gin of posterior wall and lateral wall placentae

may give rise to technical difficulties. During the
 last month before term the migration has been com-
 pleted and the lower margin of the placenta is bet-
 ter visualised. In all abnormal cases therefore the
 placental scanning should be repeated within the
 last month before term to decide the mode of deliv-
 ery.

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Tvilling graviditet?

Prognosen för tvillinggraviditet
är sämre än för enfostrig graviditet

Ca 50% av tvillinggraviditeterna
avslöjas först vid förlossningen

Förutsättning för förbättrad prognos
är att diagnosen ställs i god tid

*Screening med hCS (hPL) i 29 e och 30 e graviditetsveckan
innebär att endast ca 10% behöver efterundersökas
med exempelvis ultraljud för att fastställa duplex
Screeninggräns $> 5 \mu\text{g/ml}$ "*

1) Mägrite et al. Läkartidningen 23 (1976) 5 p. 325—326

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NEGATIVE SMEARS IN WOMEN DEVELOPING INVASIVE CERVICAL CANCER

Eva Rylander

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act Fifty six cervical smears originally classified as I from women in whom cervical cancer of stage I was detected within 4 to 5 years after the cytological check were re-examined in order to estimate the screening error and sampling failure. At the review 35 smears were classified as Pap II to V. The incidence of misclassification of the smears obtained from population screening was 70% and that of the smears taken at the specialists or at hospitals was about 50%. The incidence of sampling failure on the evaluation of the smears classified as Pap II should be considered. On 14 slides cytologically interpreted as negative there were no columnar cells present, a fact that may indicate inefficient sampling from the squamo-columnar junction.

It has been proposed that there is an average duration of about 12 years between preclinical and invasive cancer of the cervix (4). Some authors observed a high frequency of negative smears preceding an invasive cancer within a much shorter time, however (4, 11). There are various reasons for this, for instance false negativity due to inefficient sampling of material or incorrect evaluation of the cells. The trauma to the cell surface of the cervix due to vaginal washing or coitus has also been considered (8). The cancer might be situated deeper layers or endocervical cavities, thus causing poor exfoliation of cells. Certain tumours seem to have a short preclinical stage and may arise and progress between two cytological checks (1). The biological variability in exfoliation of cells from the cervix should also be borne in mind.

The purpose of this study was to find out the reasons why 56 smears belonging to women in whom cervical cancer was detected within 4 to 5 years had been classified as negative, that is Pap I smears were re-examined in order to estimate

the rate of screening error and sampling failure. The presence of low exfoliating and/or rapidly growing cancers is discussed.

MATERIAL AND METHODS

The material consists of the women invited to the cytological mass screening of the city of Stockholm in the years 1968 to 1974 in whom cervical cancer of stage I to IV was diagnosed within the same period. The patients were looked for in the registry of the Department of Gynecological Radiotherapy Radumhemmet and as a cross check also in the Registry of Cancer at the Swedish Board of Health and Welfare. Details of the way of collecting the material was described elsewhere (11).

In Sweden the triple smear containing specimen from vagina, ectocervix and endocervix is practised (14).

Out of 177 women included 64 had got at least one negative smear within 4 to 5 years prior to detection of the cancer and 5 of them had got 2 negative smears within the same time. Thus a total of 100 smears were searched for at the laboratories. Fifty six slides were obtained. Six slides had been discarded at the laboratories where they were first examined. The remaining 7 slides were not found in spite of an extensive search.

Negative smears belonging to women in whom cervical cancer or its preclinical stage had not been detected 5 years later were also added. The cytologists reviewing the smears were told about this fact but not of the number of these 7 control slides. Neither were they informed of the stages of cervical cancer detected and dates of diagnosis in the rest of the women.

Twelve of 26 smears obtained at private specialists or at hospitals and all 30 smears from the cytologic population screening in Stockholm were originally examined at the same laboratory where the review took place. Two cytologists, one cytopathologist and one cytotechnician re-examined the slides of the mass screening and the control smears. Unexpectedly it was impossible to have the remaining slides reexamined by more than one of the screeners.

Table I *The frequency of negative smears correlated to time of birth of women developing cervical cancer*

Year of birth	Number of women with cervical cancer	Frequency of negative smears	
		No	%
1914-15	32	6	19
1920-24	40	13	32
1925-29	32	10	31
1930-34	52	25	48
1935-39	21	10	48

Columnar cells were especially looked for on the slides finally interpreted as negative. The presence of this type of cell may indicate an adequate collection of material from the squamo-columnar junction in case it is situated deep into the endocervical canal.

RESULTS

The rate of negative smears in women of different age groups is shown in Table I. Negative smears were more frequent among the younger women developing cervical cancer compared to the older ones. In Table II the distribution of correctly and falsely interpreted smears is demonstrated and related to the stage of cancer detected. Twenty one smears were correctly negative, that is classified as Pap I, and 35 were judged as atypical at rescreening. Table III shows the final interpretation of smears primarily reported as negative on the one hand of those women in whom cervical cancer was detected within 4 to 5 years and on the other of the controls, that is women in whom cervical cancer had not been observed within 5 years. The frequency of incorrect evaluation of the smears taken at mass screening is as high as 70% and that of smears taken elsewhere is about 50%.

Out of 21 correctly judged smears, columnar cells were present on 7 slides only. In 4 cases of these dysplasia had been observed at an earlier check. Five patients obtained 2 negative smears within 4 to 5 years prior to detection of the cancer. In 2 of these women at least both smears were correctly negative. The time interval between the smears finally judged as negative (Pap I) and the dates of diagnosis of the invasive cancer is seen in Table IV. None of the women with correctly negative smears had got any symptoms at the date of the cytological check. Neither were there any visible changes

on the cervix. Three of the women developed adenocarcinoma of the cervix.

Adenocarcinoma was observed in 12 patients with endocervically situated squamous cancer in 177 patients of the series. Eleven of these had had a negative smear. Only six slides were examined since the rest could not be obtained. Of them proved to be incorrectly negative. At least 12 patients with the mentioned types of cancer had had atypical smears though in all cases the normality concerned the squamous cells.

DISCUSSION

Several workers noted a high percentage of negativity in cytological screening (3, 17, 18). The authors assumed this being due to a high frequency of incorrect interpretation of the smears based their statement on indirect proof only (3, 9). Pedersen, however, in re-examining 11 smears primarily classified as Pap I or II observed 10 to be falsely evaluated (10).

In the present study only smears classified as Pap I are included to the group of negative smears since they lead to no further action. Smears classified as Pap II as well as the more atypical changes give rise to follow up in one way or another. Accordingly the 56 smears of this study originally interpreted as Pap I were divided into 2 groups after rescreening: those correctly negative and those incorrectly negative. In this way the error is shown to be about 60% (Table I). Toller et al. of British Columbia noticed a similar error close to 50% in re-examining 61 smears originally judged as negative (4).

The false negativity in cytological screening of overt cancer of the cervix has been pointed out. Necrotic tissue may prevent exfoliation of malignant cells. However in the present study

Table II *The final classification of smears originally reported as negative related to the stage of cervical cancer detected*

Stage of cancer	Negative	Atypical
IA	2	4
IB	15	18
II-IV	4	13
Sum	21	35

III The final interpretation of smears originally classified as negative

valuations of 2 cytologists are reported of the smears taken at mass screening and of the control slides =
nous cells

	Cytological classification						
	SI SI	SI SI	SI SI	SI SI	SI SI	SI SI	SI SI
III of women checked at mass ing in whom cervical cancer detected within 4 to 5 years of women checked elsewhere cervical cancer was cted within 4 to 5 years	9	9	3	4	2	1	2
III of women in whom cervical er had not been observed 5 years (controls)	12	11		3			
	5				2		

changes were visible at the time of cytological
king performed at least 5 months prior to de
on of the malignancy

ie extremely high frequency of incorrect
ation of the smears taken at mass screening
) may be explained by the monotonous rou
hecking. Considering the number of smears to
reened (700 000 a year in Sweden) and the fact
98% are negative the problem is somewhat il
tated. The cytotechnicians might fail in their
tion because of the uniformity of the slides
over III certain laboratories there is a habit of
ining slides from the mass screening late in the
noon when the cytologists may be exhausted
adding some known abnormal smears to a cer
number of routine slides the watchfulness
t be tested. Also frequent changing of the type
aterial would help to counteract the monotony
duced number of routine smears per cytologist
ld raise the quality of screening

fourteen slides with normal squamous cells
olumnar cells were seen a fact that might in
e an inadequate sampling of material from the
mo-columnar junction. It could never be

proved however. Technical failure in specimen
collection contributes to complicate the interpreta
tion of the cells and may influence the classifica
tion of smears to Pap I or II. In fact sampling
inefficiency has been paid attention to as the most
important reason for false negativity and there is a
proposal always to take two smears at a time to
diminish the risk of failure in specimen collection
(13). Double sampling and checking would thus also
help to raise the correctness of evaluation of the
cells. For practical reasons 2 smears taken simul
taneously are preferable to sampling on different
occasions.

Adenocarcinoma or endocervical cancer may be
difficult to detect by cytology due to low exfolia
tion of cells or to inaccessibility. In this series a
good half of the women developing these types of
cancer had had suspicious smears. In no case were
there abnormal columnar cells present however.

Only 6 women in this series had smears with
normal squamous cells containing columnar cells as
well. It is hard to say whether in these women
there developed a cancer with a very short pre
clinical stage since a biological variation of ex
foliation of cells should also be considered.

IV The time interval between smears finally ified as Pap I and the dates of detection of the er related to stage

of ted	Time in years			
	0-1	1-2	2-3	3-4 to 5
	3	5	3	4
	1		1	1

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ULTRASTRUCTURE OF THE HUMAN PLACENTA AT TERM

Observations on Placentas from Newborn Children of Smoking and Non smoking Mothers

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701 The placenta was chosen as a possible model for studying the vascular injury and tissue injury provoked by tobacco smoking in the human body. Full term placentas from 4 smokers and 3 non smokers were studied with the transmission electron microscope. Pronounced changes were found in the group of smokers. The characteristic findings were broadening of the basement membrane of the placental villus, increase in collagen content of the villus, decrease in vascularisation and changes in the villous capillaries and arterioles with increased intimal oedema. Since similar changes have been reported previously from human umbilical arteries, since these changes also can be induced in animal arteries by exposure to carbon monoxide or perfusion with nicotine, this study supports the concept that tobacco smoking is harmful to the human vascular system and

MATERIAL AND METHODS

Patients

A group of 7 patients—4 smokers and 3 non-smokers—was examined. All patients were examined by the same investigator and each patient completed a questionnaire on smoking habits before and during pregnancy.

Non-smokers were patients who had never smoked before pregnancy and who had smoked no cigarettes at all during pregnancy. Smokers were patients who have smoked a lot before pregnancy and who still smoked. All smokers were inhaling cigarette smokers. One subject smoked 40-60 cigarettes/day.

Women suffering from hypertension, diabetes mellitus or other illness and those with Rhesus negative blood type were excluded from the material. The patients selected for the investigation were clinically healthy and normal before and during pregnancy.

All routine laboratory tests (haemoglobin, blood sugar etc.) including urine analysis for blood sugar and albumin were normal.

Oestriols in urine were not systematically examined throughout the pregnancy, but random tests revealed normal values.

The only difference between the two groups was smoking habits.

Clinical data are presented in Table I. All patients were white, had normal pregnancies and gave birth to children at term. All children were mature and no malformations were found.

The mean weight of the children in the smokers group was 3406 g, in the non smokers group 3933 g—a difference of 478 g.

The mean weight of the placenta in the smokers group was 730 g, in the non-smokers group 840 g—a difference of 170 g. Macroscopically the placentas of smokers were more fibrotic and less loose than those from non-smokers.

In the smokers group 1 boy and 3 girls were born—in the non-smoker 3 boys.

Biopsy and preparation for electron microscopy

Immediately after delivery of the placenta small blocks were cut out and placed in 4.5% cold purified

tobacco smoking during pregnancy causes complications: a higher incidence of abortion and small for date children (1, 3, 7, 12, 14, 18). Among the newborn children an increased mortality rate is found compared with children of non smoking mothers. Some investigations have even pointed out that smoking mothers are likely to have female offspring (2, 8, 12). We have recently published an investigation on a group of heavy smokers. Ultrastructural research has been performed on the umbilical artery and revealed intimal damage had been demonstrated (2). The placental weight as well as the weight of the newborn of smoking mothers is low compared with that of non smokers (2, 12). As the umbilical artery showed impressive intimal damage in the electron microscope it was considered of interest to evaluate the damage to the placenta caused by tobacco smoking.

Table 1 Clinical data

Cigs/day	Mother				Child			Placenta		
	Preg nancy number	Age (y)	Weight before preg nancy (kg)	Height (cm)	Birth weight (g)	Birth length (cm)	Sex	Weight (g)	± days to term	D. of p.
0	1	23	55	173	3 800	50	M	750	+14	<1
0	3	36	52	164	3 900	52	M	940	- 7	<1
0	1	24	60	170	4 100	53	M	850	+10	<1
10	1	17	51	170	3 450	57	F	780	- 3	<1
20	1	19	52	162	3 343	51	M	750	- 6	<1
30	4	30	60	176	3 990	54	F	780	-11	<1
60	1	31	69	175	3 250	50	F	610	0	1

glutaraldehyde containing 2% acrolein and buffered to pH 7.4 with 0.2 M phosphate buffer for one hour. Post fixation was carried out in 1% buffered osmium tetroxide (pH 7.4) for one hour at 4°C. The tissue blocks were dehydrated in graded ethanol, cleared in propylene oxide and embedded in Epon 812 or Araldite (Durocupin ACM). Sections 0.5–1.0 µm thick were stained with toluidine blue for light microscopy. Ultrathin sections were cut on glass knives with an LKB Ultratome III mounted on uncoated copper grids and contrasted with magnesium uranyl acetate and lead citrate. These sections were examined and photographed in a Zeiss 9S² electron microscope.

The present study is an extension of a previous published work on the umbilical artery. The artery was the main source for research and therefore depending on time limits for preparation only few placentas were accepted prepared for transmission electron microscopy.

The photographic work and the following description of the morphology was completed and the patients classified smokers or non smokers before breaking the code.

RESULTS

Non smokers

In the *non smokers* group the placental villus was covered on the surface by the syncytiotrophoblast often consisting of polynucleated cells. The luminal plasma membrane formed numerous microvilli. In the dark cytoplasm containing highly developed rough endoplasmic reticulum the usual organelles were present: a golgi nucleus and nucleoli, mitochondria. Glycogen rosettes were also present. The basal plasma membrane formed numerous pedicels stretching into the villous stroma (Fig. 1).

Beneath the syncytiotrophoblast was the cytotrophoblast. These cells did not always form a continuous layer of cells but often lay separately one by one.

The cytotrophoblast cells were pale with rough endoplasmic reticulum and ordinary nuclei.

The syncytiotrophoblast and the cytotrophoblast were connected by many desmosomes.

In the cytotrophoblast the basal plasma membrane often formed a small canal with connection with the nuclear envelope.

The basement membrane separated three layers of cells from the villous stroma. The basement membrane formed a continuous band with a width ranging from 40–150 nm (Fig. 1).

The villous stroma was highly vascularized, capillaries consisting of endothelium of normal permeance with tight junctions. The endothelium showed a fine fibrillary structure and beneath cells there was a thin non-continuous basement membrane.

The villous stroma contained a sparse substance with few collagen fibres and fibro-

Smokers

In the *smokers* group the same architecture was found in the syncytiotrophoblast. Loss of the luminal microvilli was a characteristic finding. There was often blebbing in the microvilli. The cytotrophoblast layer appeared flat compared with that of the non smokers due to loss of cytoplasm and there was protrusion of the nuclei into the lumen (Fig. 2). The amount of rough endoplasmic reticulum and mitochondria corresponded to that of the controls. A slight dilation of the endoplasmic reticulum due to a dark homogenous mass was occasionally found.

The cytotrophoblast layer situated beneath



Surface of human placental villus at term from a smoking mother. L Lumen S syncytiotrophoblast

V microvilli ER endoplasmic reticulum M mitochondrion BM basement membrane Bar indicates 1 μ m



Fig 2 Surface of human placental villus at term (from smoking mother day) Note the decreasing basement membrane in the syncytiotrophoblast and the increased collagen amount in the stroma. L Lumen S syncytiotrophoblast C cytotrophoblast BM basement membrane Bar = 1 μ m

syncytiotrophoblast consisted of fewer cells but there were no essential differences compared with the nonsmokers. The connection between the two cell layers was looser with loss of desmosomes.

The basement membrane was considerably widened often of width equal to that of the luminal cell layers. The syncytiotrophoblast formed long cytoplasmatic processes with extension into the basement membrane giving it a zig-zag course. The pedicels seen in the controls were not present.

An important finding was the obvious decrease in vasculatisation with loss of capillaries. The endo-

thelium in the vessels was characterized by oedema. The luminal endothelial plasma membrane was studded with blebs (Fig 3). The microvilli and closed junctions were decreased and the vessels were surrounded by a continuous broadened basement membrane often forming blocks of maternal material.

Another characteristic finding was the significant increase in collagen content in the villous stroma.

The morphological changes are presented quantitatively in Table II graded on a scale from + to +++++.



Endothelial lining in placental arteriole deriving from a placenta at term from a smoking mother (60 ciga/day). Note surface blebbing of the endothelium. L: lumen ER erythrocytes E endothelial cell B cytoplasmic process forming a surface bleb BM basement membrane Bar indicates 1 μ m

Table II *Ultrastructure of human placentas at term from 3 non smokers and 4 smokers*

The morphological changes were graded on a scale from + to ++++. The most marked differences were the villous basement membrane, the increase in amount of collagen and the poor vascularisation in the smokers. The arterioles and capillaries oedema with blebbing was seen combined with loss of tight junction in the endothelium. The formation of a continuous basement membrane. On the villous surface the number of microvilli seemed to decrease with increase in tobacco consumption.

Cigs/day	Continuous basement membrane width	Stroma amount of collagen	Stroma vascular degree	Arterioles			Circulation (in capillaries)
				Oedema (blebbing)	Continuous basement membrane	Tight junctions	
0	+	+	++++	-	-	++++	++
0	+	+	++++	-	-	++++	+++
0	+	+	++++	-	(+)	++++	++
10	+	++	+++	+	+	+++	++
20	++	++++	++	+	+	++	++
30	++++	++++	+	++	+	+	+
60	++++	++++	+	++++	+	+	+

DISCUSSION

The macroscopic, microscopic and ultrastructural appearance of the human placenta at term from normal pregnancies has been well described (6, 11, 17). The controls in this present material correspond to these descriptions. The basement membrane beneath the syncytiotrophoblast and the cytotrophoblast have not been described in detail but appear from the electron micrographs (6, 11, 17) to be comparable with the present controls. It is indicated that the average width is 150 nm (11).

No ultrastructural research in placentas delivered by heavy smoking mothers has been published to date. The present investigation on human placentas at term is an extension of a previously published work dealing with the ultrastructural changes in the umbilical artery from heavy smokers (2). That investigation (2) elicited the information that tobacco smoking causes severe intimal changes in the human vessels. Similar damage has been demonstrated in animal exposure studies (4, 10).

In the present study two particularly heavy smokers with a consumption of 30 and 60 cigarettes per day were examined. Both patients were included in the umbilical artery study (2).

The normal structure of the human placenta at term consists of the placental villi containing the capillaries with the fetal blood. The villi are covered with the later atrophying cytotrophoblast situated beneath the syncytiotrophoblast. These two cell layers form the barrier towards the maternal blood flow. The placental barrier consists of the different

layers in the capillaries, the villous syncytiotrophoblast basement membrane and the two cell layers on the villous surface. The condition and function of these layers is responsible for the fetal metabolism.

The oxygenation and the carbon monoxide uptake and exchange takes place by simple diffusion through the placental barrier (11, 13). The carbon monoxide content in the fetus depends on the mother's tobacco consumption, which varies between 2.5% and 10% (13). The carbon monoxide content of the fetal blood is a combination of cigarette smoking of the mother and the enormous carbon monoxide production in the placenta, which the first factor will result in a saturated fetal blood 1.8 times as high as that of the mother (13). The fact that oxygen saturation in the fetal circulation is low (70% oxygen saturation in the fetal vein and 26% in umbilical artery) means that additional tobacco smoking by the mother will reduce the oxygen transport and result in decreased fetal tissue oxygenation (2).

The formation of the broad basement membrane might possibly be regarded as a reparative reaction due to the low oxygen saturation. Similar changes have been found in the umbilical artery from smokers (2).

The broadening of the basement membrane has to date not been described in animal exposure studies (4, 10). It might possibly be due to a direct human reaction—or to reaction in fetal tissue.

Recently a report has been published describing broadening of the basement membrane in

tion (9) This could be a result of the increasing tobacco smoking amongst young people children and teenagers but should perhaps also be explained as a late complication of maternal smoking during pregnancy

ophoblast

Loss of cytoplasm	Basal plasma membrane polymorphism
-	-
-	-
-	-
+	+
+	++
+++	++++
++++	++++

itas at term (16) The broad basement membrane was found to be associated with the following disorders prematurity essential hypertension pre eclamptic toxæmia and diabetes mellitus. Wrapping of the membrane was seen in connection antigen/antibody reaction elsewhere in the (16) No possible correlation to tobacco consumption was mentioned

In addition in the suggested change in the fetal polymorphism caused by the broad basement membrane the essential decrease in vascularisation provided an obvious decrease in metabolism and diffusion across the placental barrier These morphological changes could explain the fact that newborn children of heavy smokers are small. In the present study and the investigation of the umbilical artery (2) have shown that tobacco smoking during pregnancy is harmful to the fetal tissue. We would expect similar changes to be found in the newborn children. Future studies must determine whether or not these changes are reversible. Among recent publications dealing with atherosclerosis there have been reports of atherosclerosis childhood calcification of the aortic valve several cases of fully developed acute myocardial infarction in children and the chaotic arteriopathy (5) Whether or not this is due to genetic factors or damage to the fetus during pregnancy is left undecided

During recent years (1972-1975) several young people (<30 years old) in Denmark have been recorded as suffering from acute myocardial infarction

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CARPAL TUNNEL SYNDROME IN OVARECTOMIZED WOMEN

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Abstract Electrophysiological parameters were studied in 32 gynaecological patients before and after major gynecological surgery. No changes in maximal nerve conduction velocity or simple reaction time could be found either after hysterectomy or ovariectomy. Of the 32 ovariectomized patients, 3 developed subjective symptoms and electrophysiological signs of the carpal tunnel syndrome within a few months after surgery. Thus the carpal tunnel syndrome, which is common in women of menopausal age, seems to be precipitated also by ovariectomy. The present findings indicate that an estrogen overproduction cannot be the basis of the carpal tunnel syndrome, but they are consistent with the idea that the syndrome is a sign of hypothalamic-pituitary overactivity or imbalance.

maximal peripheral nerve conduction velocity (NCV) of women are a little slower than those of men of equal stature if temperature and age are taken into account (6). The difference in NCV between the sexes may depend upon hormonal influences. In connection with the hormonal changes of menopause and ovariectomy there might be changes also in some aspects of peripheral nervous function, possibly even causing part of the subjective menopausal symptoms.

The present study was undertaken in order to investigate 1) whether the maximal peripheral nerve conduction velocities change as a result of ovariectomy and 2) whether estrogen therapy has an effect on nerve conduction velocity after ovariectomy. As the carpal tunnel syndrome patients are mostly women in or past the menopause, 3) the peripheral nerve segments to be studied were chosen so as to reveal 3) median nerve function changes. 4) A study of simple reaction time was included to reveal whether the possible nerve function changes are accompanied by similar changes in central nervous system functions.

METHODS

The 32 subjects were 36-54 (mean 47.1) years old and patients of the gynaecological clinic of the Turku University Hospital. They were all menstruating prior to surgery. They were divided into three groups: hysterectomy was performed on twelve patients (Group 1). On twenty hysterectomy and bilateral ovariectomy was performed. Ten of the ovariectomized patients (Group 2) received estrogen substitution therapy (2 mg of estradiol valerate *pro die*). The other ten ovariectomized patients did not receive any therapy (Group 3).

The electrophysiological measurements were made during three examinations on the day before surgery, one month after surgery but before the patients in the second group had received any estrogen therapy and finally three months after surgery.

The NCV was determined from the beginning of the first negative deflection of the averaged action potentials to 100 percutaneous supramaximal (50 V) stimulations of 0.2 ms duration in five nerve segments. The measured NCVs were: the sensory antidromic NCV of the right sural nerve at the ankle (3), the sensory antidromic NCV of the right radial nerve at the wrist (4), the sensory antidromic NCV of the right median nerve from the wrist to the base of the middle finger and from the elbow to the wrist (9), and the motor NCV of the right median nerve from the elbow to the wrist. The distal motor latency from the wrist to the thenar musculature was also noted.

The slowing of the distal sensory latency—here measured as the conduction velocity—between the wrist and the middle finger, and the slowing of the motor latency from the wrist to the thenar musculature have been shown to be the most sensitive electrophysiological indicators of the carpal tunnel syndrome (15). A marked decrease of the sensory nerve action potential in the middle finger electrodes to less than 100 µV was also noted as it has diagnostic value in the carpal tunnel syndrome (5).

Neurological signs of the carpal tunnel syndrome were also systematically looked for: Tinel's sign, wrist flexion test, hypesthesia in the four radial fingers, atrophy of the thenar musculature. If a patient complained of severe numbness or pain in her hands, electromyography of the hand musculature was performed.

The method of the reaction time study has been published elsewhere (7).

Table 1 Patients with numbness or pathological findings

n=number of any fingers α =amplitude of median sensory NAP<10 μ V β =sensory NCV of median nerve finger<40 m/s γ =median motor latency wrist-thenar<4.75 ms δ =sural sensory NCV<40 m/s radial sensory NCV<40 m/s ()=borderline value =denervation of thenar musculature **=denervation of both thenar and hyp musculature

Patient	Age (y)	Before surgery	1 month after	4 months after	Diagnosis
Group 1 Hysterectomy only (12)					
M H	43	n	—	—	None
K U	44	(γ)	—	n(α)	None
K L	44	—	n	—	None
H L	48	—	—	n *	CVII-CVIII syndrome?
Group 2 Ovariectomy + estrogen (10)					
L I	41	n	n	n	None
A V	46	n	n	—	None
L L	49	n(β)	n $\alpha\beta$	n $\alpha\beta\gamma$	Probable carpal tunnel syndrome
M P	50	n $\beta\gamma\delta$	—	—	Polynuropathy
Group 3 Ovariectomy only (10)					
S M	47	β	—	—	None
M S	48	n	n	n $\alpha\beta$	Carpal tunnel syndrome
T K	50	n	—	n $\alpha\beta\gamma$	Carpal tunnel syndrome
I P	50	n	n	n	None
A A	54	n	n	n α^{**}	CVII-CVIII syndrome?

RESULTS

There were no significant differences either between the three patient groups or between examinations 1, 2 and 3 in any measured NCV or a patient's calculated mean of all five NCVs (two way analysis of variance and *t* tests). Correction of the NCVs on the basis of skin temperatures over the nerve segment according to an unpublished method from our laboratory did not change these results. Nor did the reaction time study give any significant differences between examinations or patient groups.

However, seven patients complained of increased numbness in their hands at some examination after the surgery. Of these, three showed slowing of the wrist middle finger sensory NCV or delayed median nerve distal motor latency. The findings of all patients with complaints of numbness at any examination or with at least one pathological electrophysiological measure are combined in Table 1. The values of the measures considered pathological in the table are taken from (3, 4, 9, 15). The diagnosis in cases where it was possible to make one on the basis of the electrophysiological and clinical findings is included (Table 1).

In two cases (H L 48 and A A 54) the numbness and other findings cannot be ascribed solely to

a carpal tunnel syndrome. In both patients electromyographic denervation of both hypothenar musculature. A radical suggestion itself as they also had some neck pain. S M 50 had generally slowed NCVs and also cold extremities on examination 1. The etiology was probably severe anemia. After blood transfusion had no symptoms or signs. Also S M 50 had unusually cold extremities on examination 1.

Three patients developed a probable or suggested carpal tunnel syndrome after the gynecological surgery: two in the ovariectomy group, one in the ovariectomy + estrogen group.

Case 1 M S 48 had had some numbness of the hands from time to time for years. Before surgery there were no pathological clinical or electrophysiological findings. Neither were there any on examination 2. Three months after the ovariectomy she had increased numbness, also some night pain in her hands. Decrease of amplitude and slowing of the distal median sensory NCV with a positive Tinel's sign and denervation of the thenar musculature were the grounds for the carpal tunnel syndrome diagnosis in her right hand. She did not want more surgery, was content with only analgesics and the symptoms have declined to their former level over 1.5 years. She has had similar symptoms in her hands.

2 T K 50 had sometimes had slight numbness of hands before surgery. One month after the ectomy she was perfectly all right. Three months the surgery numbness of her right hand disturbed eep. Decrease of the amplitude of the sensory nerve potential in the middle finger and slowing of the sensory NCV and the motor distal latency of the median nerve with no other NCV changes indicated a l tunnel syndrome. Two months later she had very e left hand pain, denervation in her left median the musculature and found much relief from a left side verse carpal ligament discision. In the right hand the toms also increased and about half a year after the and operation she had one also on the right side with effect for a few weeks. Slowly returning pain indi that the right side operation has been a partial fail.

3 L L 49 had had some night numbness of her for years. On examination 1 her median distal ry NCV was slow, the borderline value. On exami 1 2 the numbness had increased and the median ry distal NCV was definitely slowed and the cor nding amplitude decreased. On examination 3 the motor latency of the right median also was slowed. ie subjective symptoms had decreased somewhat. wards she has had three periods of very severe night ness and burning pain in her right hand but has ed no other therapy than analgetics as the symptoms decreased by the time she has sought medical advice. rptoms in the left hand.

other patient in the ovariectomy+estrogen 1 had symptoms and findings suggestive of a l tunnel syndrome on examination 2 but not 1 examination 1. She is not included as her symp and findings were on the left side only and 1 no presurgery measures were determined in ffectd hand. Her symptoms subided almost etely by examination 3. e patient K U 44 in table I has also some rgs indicating median nerve dysfunction but ymptoms and clinical signs were so slight that finite diagnosis could be made.

DISCUSSION

1 nerve conduction and reaction time findings 1 ate that changes in these types of nervous m functions which are large enough to be ured by our methods do not occur after 1 ectomy at least not within a few months. The 1 ence between the NCVs of men and women 1 does not seem to depend on ovarian hormones 1. e finding does not exclude the possibility that

nerve conduction changes in slow myelinated or unmyelinated fibres form a part of the mechanism underlying postmenopausal symptoms.

Of the 32 patients at least 2 and probably 3 developed a carpal tunnel syndrome in one or both hands after major gynaecological surgery. Ovariectomy had been performed on all of these patients. Thus one of ten patients developed the carpal tunnel syndrome after ovariectomy. The full fledged syndromes developed in patients that received no estrogen substitution therapy but the differences between the patient groups are of course not statistically significant. There are no studies on the incidence and prevalence of the carpal tunnel syndrome in an unselected population of 40-50-year old women. For comparison may be mentioned that by similar electrophysiological means Leven & Huffman (8) found the carpal tunnel syndrome in one out of 48 diabetic patients and one out of 53 normals. The carpal tunnel syndrome is considered to be fairly common in diabetics (11).

Thus the present findings indicate that it is well worth while to check for symptoms of the carpal tunnel syndrome in women of menopausal age, especially after iatrogenic menopause. The cause of insomnia, numbness and pain in the hands may be this syndrome whose surgical cure is simple and effective.

The present findings also have some bearing on the etiology of the carpal tunnel syndrome. The pathophysiological mechanism of the syndrome is compression of the median nerve by the transverse carpal ligament (11). Anatomical anomalies of the median nerve or abnormalities of the wrist or after trauma or in rheumatoid disease may be etiological or predisposing factors (11). However they can hardly explain the great preponderance of women (women to men 3:1) and especially of women of menopausal age (1:11). This fact suggests the importance of female hormonal function or dysfunction for the development of the syndrome, especially as it very often is bilateral.

As the syndrome also is common in pregnant women (1:7) it has been suggested first by Wilkinson (16) that excess ovarian relaxin production may be the decisive factor. The fact that our patients developed the syndrome after ovariectomy seems to rule out this possibility.

Estrogen has been shown to have some therapeutic value in the carpal tunnel syndrome (7, 13) but contraceptive pills have been considered a cause

(12) Our results are of course inconclusive as to the effect of estrogen

Our results are consistent with an indirect effect of estrogen or other ovarian hormones (13). The loss of ovarian hormone production may cause hyperactivity or imbalance of the hypothalamic-hypophyseal system not only of FSH and LH but possibly of other hormones as well at least in some women. The role of STH or similar hormones like human placental lactogen in pregnancy and possibly even prolactin is suggested by the fact that the carpal tunnel syndrome is common almost the rule in active acromegaly (10).

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ABNORMAL URETERAL PERISTALTIC ACTIVITY DURING PREGNANCY

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Ureteral peristalsis in five pregnant patients with acute renal outflow obstruction was studied. In addition a new technique for recording intraureteral (ureterometry). With this technique it is possible to record the intraureteral pressure at three different levels of the ureter simultaneously. The rate of propagation of the peristaltic waves can thus be determined. Retrograde peristalsis was found in three of the patients and in one patient no peristaltic activity was found at all. The fifth patient demonstrated normal peristalsis. The findings at renography in the five patients were in accordance with the ureterometric

transport of urine from the renal pelvis to the bladder is due mainly to ureteral peristalsis. This phenomenon which is of great interest to the urologist. It is well known that pregnancy as well as tumours of the reproductive organs can interfere with the transport of urine by compressing the ureter (3, 4, 7, 9, 10, 14, 15). However ureteral peristalsis is a characteristic of pregnancy may also be the effect of progesterone and the effect on peristalsis may not be due solely to extrinsic pressure

Ureteral activity is usually studied with urography. This method however gives only instantaneous pictures of ureteral function. Cineradiography of the ureters gives more detailed information about ureteral peristalsis but opinions differ on the clinical significance of such information (5, 6). Furthermore such an investigation technique cannot be used during pregnancy.

Ureteral peristalsis can also be studied for a longer time with the aid of intraureteral pressure recordings (5, 6, 10, 11). Such investigations have to be performed with a conventional ureteral catheter connected to a pressure transducer. However when using only one catheter the in-

traureteral pressure is recorded at one level only and no information about the pressure amplitudes at other levels of the ureter is obtained. Hence it is not possible to calculate the direction and propagation rate of the peristaltic waves. In addition the use of a conventional ureteral catheter with a side hole involves also the risk of the ureteral mucosa plugging the aperture of the catheter with an incorrect recording as a result (5, 6, 12).

To avoid these disadvantages a new technique for recording intraureteral pressures (ureterometry) has recently been devised (12). With this technique it is possible to determine the rate of propagation and direction of ureteral contractions along the ureter.

In a ureterometric study of healthy women with this technique it was observed that the normally antegrade direction of ureteral peristalsis was sometimes transiently (<1 min) reversed (12). These short episodes of retroperistalsis were not accompanied by any discomfort to the patients. In a following study of the ureters with combined cineradiography and ureterometry it was noted that longer periods of retroperistalsis (>15 min) sometimes occurred in patients with genital tumours (15). The peristaltic waves then travelled towards the kidney and prevented the urine from reaching the bladder. This resulted in a functional ureteral stasis with a high intrapelvic pressure and the patients then complained of pain in the flank (15).

Ureteral dilatation is a common phenomenon in pregnancy. In most cases it is asymptomatic. In some patients however it is combined with episodes of severe pain in the flank. In the absence of mechanical obstruction such as ureteral stones the cause of combined ureteral stasis and suddenly marked pain is obscure. It was therefore thought to

Table 1 Relevant data of the five investigated patients

Pat No	Age	Month of gestation	Renography	Side of pain
1	27	Pregnant mens VIII	Obstruction right normal left	Right flank
2	26	Pregnant mens VI	Obstruction right normal left	Right flank
3	27	Pregnant mens V	Obstruction right normal left	Right flank
4	28	Pregnant mens VII	Obstruction right normal left	Right flank
5	32	Pregnant mens IX	Normal bilateral	Both flanks

be of interest to perform a ureterometric study in pregnant patients admitted to the hospital because of pain in the flank due to suspected acute renal obstruction. The question was whether retrograde ureteral peristalsis was common in these patients.

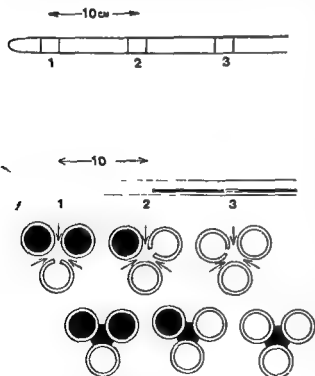


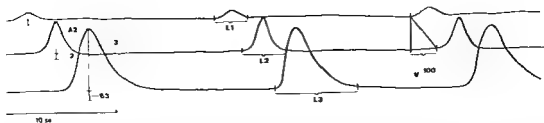
Fig. 1 Catheter used for transmission of intraureteric pressures. Pressure is transmitted from three points 1, 2 and 3 which are 10 cm apart. The lower part of the figure shows cross sections of the catheter at every recording section and between these sections. The black colour indicates glue. Note the possibility for urine to flow into the measuring aperture of the relevant channel. Due to the construction of the catheter ureteral mucosa cannot interfere with the measuring aperture.

MATERIAL

Five pregnant patients in the fifth month of pregnancy were examined (Table 1). All patients were pregnant for the first time and all denied previous urological tract. The pregnancy was uncomplicated until the appearance of the actual flank pain which had continued for some hours. In four cases the pain was also combined with dysuria. All patients were examined with no complications from the uterus were found. Microscopic examination of urine revealed nothing remarkable. No haematuria. During the examination the most striking finding was the marked tenderness in the right flank in four of the patients. The preliminary diagnosis was in four cases acute renal obstruction and the patients were referred to the renal department. Due to the result of this examination (Table 1) and the severe pain—in some patients unlike renal colic—it seemed justified to perform a cystoscopy including passage of a catheter in order to exclude mechanical obstruction. study ureteral peristalsis at the same time a recording catheter was used instead of a conventional catheter.

Recording technique

The ureterometric investigation technique has been described in detail elsewhere (12). It is therefore only briefly outlined here. The intraureteral pressure was measured by means of a specially designed multichannel catheter (Elema 741) connected to pressure transducers (Elema Siemens 81). After amplifying (Elema Siemens 81) the pressure signals were recorded by an ink recorder (Elema Siemens 81). As the intraureteral pressure was recorded at three levels simultaneously it was possible to determine the rate and amplitude of the penstaltic waves. Fig. 2 gives a normal intraureteral pressure diagram obtained with this recording technique. The calibration procedure which included no pressure transducers but also the recording technique is described elsewhere (12).



Normal ureteral contractions recorded at three levels in the ureter 1 2 and 3 A=amplitude cm basal or resting pressure cm H₂O L=duration of 100 sec V=rate of propagation mm/s The up-tracing gives the intraluminal pressure at the up-

permost part of the ureter near the renal pelvis the middle tracing at the middle part of the ureter and the bottom tracing at the terminal part of the ureter As a rule the recordings are made 25 15 and 5 cm from the urinary bladder respectively

ental procedure

aid of a Wolff ureterocystoscope the recording was placed in the ureter with the tip of the catheter in the renal pelvis. This excluded mechanical obstruction. The lowermost recording section of the catheter was fixed 5 cm from the ureteral orifice. This means that the construction of the catheter (Fig. 1) the ureteral pressure was recorded simultaneously 5 15 cm from the bladder. During the recording the ureter was empty. The ureterometry was undertaken on the patient as described by the patient as painful and showing in the renogram (Table I). When possible ureterometry was also undertaken on the other side. In some of the patients complained of severe pain these cases the investigation could not from an point of view be prolonged to include also the other side. The recordings covered 20–30 minutes were not repeated as repeated passage of a catheter into the ureter would presumably have increased the risk of urinary infection especially in these patients. All patients received antibiotics ampicillin 2 g daily for 14 days (Astra Sweden). Renography was done and cultured immediately before and several times after the recording procedure.

Conclusions and interpretations of the records

The uppermost tracing of the pressure diagrams (Figs 2 and 3) as recorded by means of the uppermost pressure recording section of the catheter (Fig. 1). As described this part of the catheter was placed 25 cm from the ureteral orifice and was thus situated just below the renal pelvis. The middle and the lowermost tracings were recorded from the middle and the lowermost pressure recording sections of the catheter thus presenting the pressures from the middle and lower part of the ureter respectively. The amplitude of the pressure change is the difference between the peak pressure observed during the ureteral contraction and the resting pressure between these contractions. The rate of propagation (V mm/sec) is determined from the time taken for the contraction wave to travel from one section of recording to the next. The direction of peristalsis (antegrade or retrograde) can be determined by observing

at which point of recording the contraction wave first appears. The frequency (F) is the number of contractions per minute.

RESULTS

Table I gives the results of the renographies done in the acute stages. Except for patient 5 all renograms demonstrated obstruction on the affected side.

From Table II which gives the ureterometric data from the patients it is clear that retrograde peristaltic waves were present in three of the five patients. The retroperistalsis persisted in these patients throughout the recording period (>20 min). Figs 3a and b give pressure recordings from one of these patients (no 1 Tables I and II). Fig 3a demonstrates that extensive retrograde ureteral peristalsis was present on the right side whereas Fig 3b shows normal antegrade peristalsis on the left side. As can be seen from Table I the renogram in this patient demonstrated ureteral stasis on the right side but not on the left.

The resting pressure i.e. the basic pressure between contractions were higher at all recording levels in the abnormal right ureter compared with those in the normal left. The most striking discrepancy in this respect was present at the highest recording level near the renal pelvis where the resting pressure was almost three times as high in the right ureter as in the left. The patient also complained of pain in the right flank (Table I).

The rate of propagation as well as the peristaltic pressure amplitude at different recording levels was about the same as those given previously (12, 15).

The ureterometric findings in patients 2 and 3 (Table II) were about the same as those

Table II Ureterometric data of the five investigated patients

Pat No	Ureter examined	V=peristaltic travel rate (mm/s)	F=contractions/min	Direction of peristalsis	Peristaltic pressure amplitude and (Resting pressure) in cm H ₂ O at distances from bladder indicated	
					5 cm	15 cm
1	Right	40	7	Retrograde	30 (10)	25 (15)
	Left	30	4	Antegrade	25 (6)	15 (7)
2	Right	40	6	Retrograde	32 (12)	25 (15)
3	Right	35	6	Retrograde	20 (10)	20 (15)
4	Right	—	—	—	— (15)	— (15)
	Left	30	4	Antegrade	22 (8)	25 (10)
5	Right	35	7	Antegrade	25 (8)	20 (9)
	Left	30	6	Antegrade	28 (8)	20 (9)

sumably because of the lack of a suitable technique for demonstrating the direction of the ureteral contractions. When using two conventional ureteral catheters for intraureteral pressure recordings, Kul sometimes recorded retrograde peristalsis (5). It has then been claimed that the presence of two rigid conventional ureteral catheters (size 5 F) (I F=0.33 mm) may obstruct the urinary flow and disturb the normal activity of the ureter. The present investigation was undertaken with a very thin and flexible catheter. Its outer diameter is only 4 F=1.3 mm. It has previously been confirmed with combined ureteral pyelography and ureterometry that this catheter does not produce ureteral stasis in healthy pregnant women (15). If the presence of the recording catheter was the main reason for the peristalsis it should have occurred also on the unaffected side.

The retrograde contractions in the present investigation originated entirely from the lower part of the ureter where recently numerous adrenergic and cholinergic nerve-cells have been demonstrated (11). It is conceivable that the activity in these nerve-cells under certain circumstances can influence the automaticity of latent pacemaker cells in this part of the ureter and thereby interfere with the activity of the normal pacemaker cells situated in the upper part of the ureter or renal pelvis. In a previous ureterometric investigation before and during and after Wertheim hysterectomy it was noticed that retrograde peristalsis occurred during dissection of the lower part of the ureter (13). When manipulating the lower part of the ureter in dogs Enhorning & Weaver also elicited retrograde peristalsis (2).

It has previously been demonstrated that episodes of retrograde ureteral contraction produced a functional ureteral stasis. When the traction waves travelled towards the kidney prevented urine from reaching the bladder caused a rapid increase in intrapelvic pressure. As seen from the ureterometric data (Table II) intraureteral resting pressures were present at recording point close to the renal pelvis in all patients with retrograde peristalsis.

It is now generally accepted that flank or renal colic pain in cases of ureteral obstruction is due to a sudden increase in the intrapelvic pressure. It is therefore possible that periods of retrograde peristalsis can contribute to the explanation of the marked pain and pronounced ureteral stasis sometimes occur in pregnant patients. In the present investigation only five patients were examined, of them may be considered healthy from a clinical point of view. It is therefore not quite correct to compare the ureterometric data of these patients with those obtained in normal non-pregnant volunteers (12). For several reasons—above all, clinical—we have not examined urological healthy pregnant patients with our ureterometric technique. However, investigations of pregnant patients without signs of ureteral obstruction have been performed by Ruby & Sala (9, 10). In these investigations a conventional ureteral catheter with only one measuring aperture was used. It has been questioned whether such a technique permits certain conclusions about ureteral function at different levels in the ureter. To do this it is necessary to perform simultaneous pressure recordings at different levels in the ureter (5, 6, 17, 18). It

normal antegrade peristalsis was demonstrated. Our data are about the same as those recorded by Ruby & Sala (9-10). These authors suggest that the dilatation of the ureters during pregnancy is due mainly to mechanical obstruction. The results of this investigation may also support this suggestion. For it has been claimed that the first sign of mild ureteral obstruction is an increase in the resting pressure and frequency of contractions (1-2, 5-15). In this investigation also in women with normal antegrade peristalsis the intraluminal pressures were higher and the frequency of contractions increased compared with those recorded in healthy volunteers (12).

The management of pregnant patients with severe ureteral stasis is delicate. The passage of the double-J catheter into the renal pelvis in our patients excluded mechanical obstruction. The patients were then treated symptomatically with papaverine 80 mg/day (Papaverine ACO, Sweden). They were instructed to lie on the unaffected side. All patients were free from their acute pain within 20 hours.

Renograms after one week demonstrated no change in the obstruction on the affected side. However, the renograms were not normal until two weeks after the delivery. All five patients were delivered at full term without any complications. Radiographs obtained from all the patients two weeks after the delivery were normal. There were no signs of urinary infection in these patients as the results of microscopy as well as repeated urine cultures were normal. However, all the patients were given antibiotics. During the first 14 days after the examination the women received ampicillin 2 g/day (Ampicillin Astra, Sweden) and in the following 14 days furazolidone 150 mg daily (Furadantin, Pharmacia, Sweden) throughout the pregnancy.

The side effects of the ureterometric investigation were noticed. It must however be emphasized that passage of a catheter into the ureter always involves risks. One must therefore have a clear indication for such a manoeuvre and in our case the patient should receive antibiotics during and after this procedure.

Further examinations should be avoided during pregnancy. In suspected ureteral obstruction passage of a catheter into the ureter might therefore sometimes be necessary. In such a situation it is important to collect as much information as possible about ureteral function. The present recording

catheter seems superior to conventional ureteral catheters. It gives information not only on the presence of a mechanical obstruction but also detailed information about ureteral peristalsis.

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CYCLIC FLUCTUATION IN NORADRENALINE TRANSMITTER OF THE MONKEY OVIDUCT

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Cyclic variations in noradrenaline of the sympathetic nerves (short adrenergic neurons) innervating both musculature of the oviduct have been determined by fluorescence histochemistry and fluorometric measurements in monkeys (*Macaca mulatta*). During the estrous phase there is more than twice as much noradrenaline in the oviduct compared with the proliferative phase. This suggests that the system of adrenergic nerves in the primate oviduct is involved in the motility associated with alterations in the level of endogenous estrogen and progesterone during the menstrual cycle.

It is generally believed that the smooth musculature of the oviduct plays an important role in the transport of the ovum from the ovary towards the uterus. The detailed mechanisms involved in this contraction are not very well understood (24, 33). There is much experimental evidence to indicate that the motor activity of the tubal smooth musculature is under adrenergic influence mediated by receptors associated with excitation and inhibition, which are inhibitory (3). Under the influence of hormones there is a functional balance between these receptors so that in essence progesterone potentiates the α receptor response, whereas the response to β receptor stimulation is inhibited by progesterone (13). Fluorescence histochemistry has demonstrated that the oviduct possesses a well-developed adrenergic innervation related to the smooth musculature (26, 30). The activity of the adrenergic nerves in the oviduct can be affected by sex steroids administration. Estrogen has been found to increase the level of noradrenaline, which becomes normalized after the influence of progesterone given along with the estrogen (30).

The present study on monkey oviducts was undertaken to find out whether such fluctuations in the noradrenaline transmitter can be revealed also during the normal endogenous hormone changes associated with the menstrual cycle.

MATERIAL AND METHODS

The material consisted of 13 adult normally menstruating rhesus monkeys (*Macaca mulatta*). The cyclic stage was determined in either of the following ways: (a) relation to the first day of last menstrual bleeding; (b) histological examination of the endometrium (formalin fixed material stained in hematoxylin and eosin) and (c) daily radioimmunoassay of estradiol 17 β and progesterone in plasma (10, 37). One or both oviducts were removed during nembutal anaesthesia. For the fluorescence histochemical demonstration of noradrenaline (2) two of the tubes, also containing the tubo-uterine junction, were used for serial sectioning. Only small pieces from the isthmus, 2 cm from the tubo-uterine junction, were taken from the remainder of the oviducts. The tissue pieces were frozen to the temperature of liquid nitrogen, freeze-dried, treated with formaldehyde gas at +80°C for 1 hr, sectioned at 6 μ and mounted in Entellan (Merck) for fluorescence microscopy (2).

Apart from the small pieces taken for the histofluorescence examinations, the whole oviducts from 11 of the animals were homogenized in 0.4 N ice-cold perchloric acid for chemical determination of noradrenaline (1, 12). Either one or both oviducts were used in each determination.

RESULTS

In all parts of the oviduct fluorescence microscopy showed the presence of numerous adrenergic nerve terminals emitting a green light under the optical conditions used (2) and characterized by a beaded

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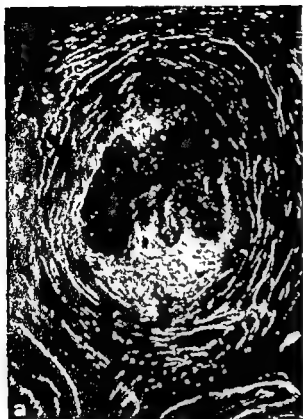


Fig 1 Fluorescence photomicrographs of transversely sectioned oviducts isthmus region. The majority of fluorescent adrenergic nerve terminals run in the circular muscle layer in the same direction as the smooth muscle cells. The number of fluorescent axon terminals visible

during (a) the secretory phase of the menstrual cycle was much larger than (b) during the proliferative phase. This is in agreement with the chemical detection of noradrenaline (cf Fig 2 insert) $\times 125$

variance. Most of the fibres were distributed in association with the smooth muscle cells. In the same direction they followed in the circular and longitudinal layers. The remainder of the fluorescent nerves ran along vessels both in the muscle layers and in the lamina propria of the mucosa. In addition the lamina propria contained some adrenergic nerve terminals without clear relation to blood vessels.

In the serially sectioned material it could be shown that in the ampulla the thin muscle layer received only relatively few adrenergic nerves. The number of fluorescent fibres showed a distinct increase in the isthmus where the prominent circular muscle layer was particularly well supplied (Fig 1). The number of nerves in this layer was maintained throughout the entire length of the isthmus. The picture was similar also in the tubo-uterine junction whereas the smooth musculature in the adjacent part of the uterus had a lower density of adrenergic nerves compared with the isthmus.

On the basis of microscopic analysis of sections from the isthmus it was possible to demonstrate a clear cyclic variation in the fluorescence picture of the adrenergic nerve terminals most evident in the circular smooth muscle layer. Thus during the proliferation stage the number of fluorescent nerve terminals was moderate and fluorescence was less bright than during the secretory phase when the fibres were more numerous (Fig 1). With regard to the fairly short time periods involved it is conceivable that this does not represent a true variation in the number of adrenergic nerves but rather a change in noradrenaline concentration of the individual oviducts which means that the transmitter level is low for histochemical detection in many oviducts during the proliferative phase.

As shown in Fig 2 oviducts from the proliferative phase were obtained at cyclic day 4-6 when the mean plasma estrogen in this phase was 78 pg/ml, somewhat lower than during the secretory phase.

THE SUPPRESSION OF PUERPERAL LACTATION WITH BROMOCRYPTINE

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Abstract. The therapeutic value of 2-Br- α -ergocryptine for suppression of puerperal lactation was studied in 30 women. 16 received the drug, 14 were controls. In women plasma levels of FSH, oestradiol and alpha-lactalbumin were measured in labour and in the early puerperium. Brom-ergocryptine was found effectively to suppress milk production and alleviate breast pain and engorgement with no side effects and minimum rebound on prolactin. The inverse relationship between prolactin and levels reported elsewhere in non-pregnant women appears to occur in the early postpartum period. Although there was a significant rise in alpha-lactalbumin in labour and the puerperium over non-pregnant women there was no difference between lactating and non-lactating women.

Recent attempts to inhibit lactation were limited to mechanical compression of the breasts, restriction of fluid intake supplemented by diuretic agents which are ineffective and hormonal inhibition by oestrogens either alone or in combination with progesterone and androgens which were effective but found to increase the risk of thrombo-embolic complications in the puerperium. Research for a non-hormonal compound specifically inhibiting prolactin release and suppressing lactation led to the selection of the ergot alkaloid α -ergocryptine (CB 154).

Preliminary studies on animals by Flückiger & Wagner demonstrated the inhibition of lactation by α -ergocryptine. Based on these findings the drug was used by Lutterbeck et al. (8), Besser et al. (9) and others for the treatment of non-puerperal mastitis and by Varga et al. (15). Rolland et al. (11, 12) and others for the suppression of puerperal lactation.

The present study of 30 women was undertaken to evaluate the clinical effectiveness of Bromo-

ergocryptine in suppressing lactation and to observe any side effects over a period of 28 days. In six women three on the drug and three controls the plasma FSH and oestradiol levels were estimated as a measure of the effect of the drug on pituitary-ovarian function since it has been demonstrated that in the non-pregnant individual there is an inverse relation between prolactin and gonadotrophin secretion (2). Estimation of plasma lactalbumin levels was used as an additional parameter of mammary gland activity (6).

METHODS AND MATERIAL

Women who elected not to breast feed were selected antenatally after the 36th week of pregnancy. The plan of study was explained to them and a signed consent obtained. Only women who had a normal pregnancy and a vaginal delivery were included. Special note was made of any concomitant treatment with diuretics, phenothiazines or steroids because of the possible effect of these drugs on lactation. No alternative methods to suppress lactation were adopted and analgesics were given if required.

Women were selected and the tablets allocated double blind except in the six women who volunteered to have blood samples taken who were selected by the statistician controlling the trial to give three in each group. In the remaining women the code of the group was not revealed to the investigators until the study was completed. Each active tablet contained 2.5 mg of the active compound 2-Br- α -ergocryptine. Two tablets (5 mg) were given daily after the main meal usually at midday for the first two weeks and one tablet (2.5 mg) daily for the third week. Treatment began within twelve hours of delivery. The efficacy of suppression of lactation was assessed by observation of milk production, congestion and pain in the breasts or arbitrary scales similar to those described by Rolland & Schellekens (11) (Table 1).

Assessment was made at the same time each day based on subjective evaluations by the women who were instructed and supervised by a Research Sister.

Table I Determination of mammary activity

Score	Milk production by palpation	Congestion	Pain
0	No milk	Absent	Absent
I	Some drops	Mild	Mild
II	Slight outflow	Moderate	Moderate
III	Stream of milk	Severe	Severe

Requiring analgesics

stay in hospital. The observations were entered on score cards which the women maintained for a period of 28 days after delivery and which were then returned in self-addressed envelopes. Women were finally assessed at the postnatal visit six weeks after delivery. All general practitioners were requested to inform us should they be called upon to treat any side reactions or if the drug was discontinued due to other medication.

In the six women who volunteered for detailed hormone studies 10 ml of heparinised blood was collected in the first stage of labour, after the third stage of labour and on alternate days while they were in hospital. Plasma FSH, oestradiol and alpha lactalbumin was estimated by radioimmunoassay. FSH was assayed by the double antibody radioimmunoassay method of Reuter, Gaspard and Franchimont (10) using the FSH Kits produced by CEA-IRE SORIN. The reference standard for FSH used was of pituitary origin supplied by Calbiochem. Its biological potency is 3500 IU/mg (IRP2 HMG). Equivalence of reference standard/International standards -1 mg Reference Hormone = 169 ± 19 mg LER 907 or 2830 ± 170 IU 68/39 MRC Mill Hill.

Plasma oestradiol was assayed by the method described by Bolton & Rutherford (3).

Plasma alpha lactalbumin was measured by a double antibody radioimmunoassay using antisera raised against human alpha lactalbumin prepared by the method of Arman (1). This material was also used for radioiodination and for assay standards.

RESULTS

A total of 44 women were recruited. Eight were lost sight of and failed to return their cards; in six others

treatment was discontinued for various reasons related to the use of the drug. Thirty women completed the study. 16 on bromo-ergocryptine and 14 on placebo. Complete records were maintained for 20 women during the first two weeks, two women did not complete the record in the third week, seven women on the drug and six controls failed to maintain the cards in the 4th week. The records were rechecked with the women at the postnatal visit.

Statistical analysis of the effect of the drug on milk production, breast congestion and pain was judged by the proportion with negligible symptoms given in Table II. Table III shows the proportion with more severe symptoms.

Moderate to severe pain was noticed in 7% of controls as against 19% of patients treated with Bromo ergocryptine in the first week. No pain was reported after the second week in women treated with the drug.

No adverse reactions or side effects were observed in either group. At the postnatal assessment six weeks after delivery menstruation was re-established in 5/9 women on the drug and 10/14 women on the placebo. Eleven women failed to keep their hospital postnatal appointment. A recurrence of lactation was reported by one woman in the bromo-ergocryptine group who developed galactorrhoea six weeks postpartum and one woman after menstruation was re-established but it regressed without further treatment.

Plasma was obtained from six women in the controls and three on the active drug. Levels of plasma FSH, oestradiol 17β and alpha lactalbumin are given in Table IV and in Fig. 1 the alpha lactalbumin results are compared with the results of lactating women. The mean alpha lactalbumin level in a non pregnant non puerperal group was found to be less than 10 ng/ml.

Table II Proportions of subjects with total weekly score equal to 0 or 1

Week	Milk production		Congestion		Pain	
	Active	Placebo	Active	Placebo	Active	Placebo
1	12/16 75%	1/14 7%*	13/16 81%	1/14 7%	13/16 81%	3/14 21%
2	12/16 75%	2/14 14%*	14/16 88%	6/14 43%	14/16 88%	11/14 79%
3	14/15 93%	6/13 46%*	15/15 100%	13/13 100%	15/15 100%	11/13 85%
4	9/9 100%	3/8 38%	9/9 100%	8/8 100%	9/9 100%	7/8 88%

* Statistically significant $P < 0.1$ * Statistically significant $P < 0.5$

ough a marked increase in alpha lactalbumin were observed in labour and the puerperium does not appear to be any systematic difference in levels observed in lactating and non-lactating women although those in women on bromocryptine tend to be somewhat lower. The number of cases studied is too small for statistical analysis.

DISCUSSION

Earlier studies of bromo-ergocryptine side effects like heartburn, anorexia, vomiting and gastrointestinal symptoms were reported (2, 3) but the study of bromo-ergocryptine has been shown to be effective in suppressing lactation without ill effects. In addition, the dose schedule over a period of 4 weeks—5 mg daily for the first and second week and 2.5 mg daily for the third week—almost eliminated the problems of rebound lactation reported in an earlier publication.

The inhibitory effect of bromo-ergocryptine in suppressing prolactin release in the galactorrhoea syndrome and in puerperal lactation has been documented. Rolland et al (11) demonstrated that prolactin concentrations were effec-

Table III Proportion of patients showing moderate to severe pain (scores 3 and 4)

Week	Active		Placebo	
I	3/16	19%	11/14	79%
II	2/16	12%	3/14	21%
III	0	0	2/13	15%
IV	0	0	1/8	12%

tively reduced by bromo-ergocryptine after delivery and FSH was scarcely detectable during late pregnancy and the early postpartum period. Our findings of negligible levels in the first ten days after delivery are in agreement. The high levels of oestrogen characteristic of pregnancy were seen in samples obtained in early labour and soon after the third stage but showed the usual dramatic fall within 48 hours of delivery to low levels which were maintained through the first ten days in both groups of women. The inverse relationship between prolactin and FSH observed in the non-pregnant woman (2) does not appear to relate to the early postpartum period as is evident by a failure in FSH levels to rise after prolactin suppression by bromo-ergocryptine. These findings are confirmed by Del Pozo et al (4) who demonstrated a significant in-

Table IV Plasma levels of FSH (ng/ml), oestradiol 17 β (ph/ml) and α -lactalbumin (ng/ml) in 3 treated and 3 untreated women

	Early labour	Third stage	48 hrs	96 hrs	144 hrs	192 hrs	
oestradiol	0.25	0.27	0.25	0.25			
albumin	44.000	29.200	37.5	40.5			
		37 (0)	22 (0)	25 (1)			
oestradiol	0.20	0.24	0.23				
albumin	16.600	8.700	104				
		220 (0)	190 (0)				
oestradiol	0.26	0.1	0.1	0.1	0.26	0.25	
albumin	28.400	12.200	91	40	22	29	
	755 (0)		243 (2)	>300 (2)	>300 (2)	270 (1)	
oestradiol	0.5	0.26	0.28	0.25	0.26	0.23	0.52
albumin	49.000	34.700	590	67	34	43	
		180 (0)	10. (0)	71 (0)	140 (0)	>300 (0)	
oestradiol	0.5	0.24	0.25	0.25			
albumin	6.600	4.600	37	29			
	36 (0)		13 (1)	10 (1)			
oestradiol	0.25	Too low	Too low	Too low		0.25	
albumin	Not done						
	Not done						

SHORT COMMUNICATION

IN VIVO SUPPRESSION OF UTERINE LYMPHOCYTES DURING
EARLY HUMAN PREGNANCY

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immunological acceptance of the foetus being allogeneic homograft is still a perplexing phenomenon. How can the foetal trophoblast enter into the endometrium and survive while exposed to the maternal immunological defense? It has been postulated previously that a mucoprotein coating trophoblastic antigens (1) or an anatomical foetomaternal separation (2) should be essential in the engrafting process. A number of soluble substances have also been suggested to be responsible for this successful transplantation by inhibition of maternal lymphocyte transformation. Thus in previous studies we have demonstrated that human chorionic gonadotropin (HCG) (3-6), human chorionic somatomammotropin (HCS) (4) and prolactin (7) inhibit lymphocyte transformation as measured by different antigens and mitogens. In addition

human IgG possibly locally produced and eluted from placental tissue has been shown to possess a similar inhibitory effect (8, 9). The underlying mechanism is still unclear but these antibodies are most likely not directed towards any as yet specified surface component since antibodies directed against known T or H cell antigens do not significantly inhibit the mitogen induced proliferative response of lymphocytes.

During pregnancy a factor has been demonstrated in maternal plasma that suppresses the reactivity of peripheral blood lymphocytes (10, 11). As this factor is probably produced by the placenta the lymphocytes adjacent to the uterus should be functionally more suppressed than those in the peripheral circulation.

To elucidate this postulation lymphocytes were

Table 1 Mitogenic response of human lymphocytes from peripheral or uterine blood after 3 days of culturing in serum-supplemented cultures (150 000 cells/culture)

Patient	Week of gestation	PHA response (mean of triplicates)			Rabbit anti human β microglobulin (mean of triplicates)		
		Peripheral lymphocytes	Uterine lymphocytes	Significance (Student's <i>t</i> test)	Peripheral lymphocytes	Uterine lymphocytes	Significance (Student's <i>t</i> test)
	8	53 416	2 408	$p \leq 0.001$	6 733	490	$p \leq 0.001$
	9	110 454	5 877	$p \leq 0.001$	17 709	53	$p \leq 0.001$
	9	78 414	60 405	Not signif.	14 243	3 659	$p \leq 0.001$
	17	30 985	8 606	$0.05 > p > 0.001$	2 474	930	Not signif.
	9	81 649	58 078	Not signif.	13 308	11 509	Not signif.
	8	80 438	58 395	$0.05 > p > 0.001$	12 408	4 061	$p \leq 0.001$
	9	91 510	67 765	$0.05 > p > 0.001$	801	475	$0.05 > p > 0.001$
	9	114 838	9 087	$0.05 > p > 0.001$	7 817	3 0 6	$0.05 > p > 0.001$
	9	15 997	173	$p \leq 0.001$	4 669	198	$p \leq 0.001$

lythae magglutinin

CASE REPORTS

SPONTANEOUS RUPTURE OF THE UTERUS IN THE THIRD TRIMESTER WITH A LIVING FETUS EXPELLED INTO THE ABDOMINAL CAVITY

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CASE REPORT

The woman, aged 37 and according to last menstrual period 33 weeks pregnant, was admitted because of abdominal pain. In 1963 she had had a normal delivery. In 1971 she aborted spontaneously in the third month of pregnancy and a curettage was performed with a blunt curette. The recovery was uneventful. Macroscopic examination revealed necrotic chorionic tissue but no villi or myometrial fragments.

She had been well until six days before her admission in January 1972. During these days she had experienced intermittent abdominal pain increasing at night and causing loss of sleep.

When admitted to hospital her general condition was unaffected and she had no signs of toxemia. The abdomen was soft, with no tenderness. The uterine fundus was halfway between the umbilicus and the xiphisternum and its wall was slightly tense. The fetus had a longitudinal lie and a cephalic presentation with the head just over the pelvic inlet. The fetal heart rate was within normal limits. The cervix was firm and not effaced or dilated and the vagina contained no blood.

As premature labor seemed possible Isosuprine was administered intravenously. The abdominal pain subsided, but on the following day the patient—still apparently well and afebrile—complained of stabbing abdominal pain and she occasionally vomited. While the uterus was very tense and tender—especially so fundally—and the fundal shave had grown asymmetrical, the patient admitted insignificant tenderness of the abdomen which seemed soft. Fetal heart rate was ~136. She was given analgesics as well as Terbutalin with some effect.

On the third day after admission the patient had occasionally abdominal pain at infrequent intervals and her general condition was still good. There was moderate tenderness in the epigastric region extending further down in the abdomen. The uterus seemed as large as before and rather soft. The lie of the fetus had changed to transverse. Fetal heart sounds could no longer be detected.



Fig 1 Abdominal surveyfilm, lateral view. High and transverse lie of the fetus surrounded by gas-distended loops of the large and small bowels

During the following two days the symptoms became more severe and in particular the colicky pains. The patient grew rather pale. She was still afebrile and had normal or slightly elevated blood pressure and a pulse rate of $\sim 100/\text{min}$. The abdomen had become distended and tympanic. Radiological examination of the abdomen beginning on the fourth day revealed a fetus lying transversely at the umbilical level and a severely gas-distended colon (*Fig 1*). Barium contrast enema failed to disclose a low placed obstruction of the colon (*Fig 2*).

Attention was considered necessary. The laparotomy view of the patient's state and the findings surgical attention was considered necessary. The laparotomy revealed that the greatly distended sigmoid concealed the other abdominal organs. A small amount of blood was present in the cavity which in its upper part was taken up by the fetus with its head against the spleen. The infant was dark blue and had a low muscle tonus, the Apgar score being ≤ 3 . The umbilical cord was immediately cut and adequate resuscitative steps taken. The infant soon recovered, grew pink and started to cry. Through a transverse tear of the uterine fundus the placenta was partially expelled (*Fig 3*). Due to the extensive damage to the wall a subtotal hysterectomy was performed.

Microscopically the uterine wall at the site of the rupture showed signs of marked non-specific inflammation with degenerative changes of the myometrium. No further pathological changes were observed as far as the placenta and uterus were concerned.

As regards the child, the birth weight being 2600 g and the length 45 cm, its arterial blood pH was 6.8 initially. The acid-base data returned slowly to the normal range. So far its development has progressed normally. The post-operative course of the mother was uneventful.

DISCUSSION

The clinical symptoms and signs are explained by the following sequence of increasing uterine activity: the tensile part of the fundal wall eventually bro small rupture, the edges of which separate slowly. Between the 2nd and membranes ruptured. Thereafter the expelled into the abdominal cavity the lie. The uterine activity must then ha considerably and the placenta rema a result of which the uteroplacental kept adequate for nutrition of the fetus tis—and thereby paralytic ilr uterine contents in the abdominal cavity. time of laparotomy the uterine activity causing separation of the placenta which partially expelled through the tear (*Fig 3*). the utero-placental circulation rapidly bringing about an acute and asphyxia.

The radiological signs characteristic of rupture have been described by Zupp Parkinson (1958) and Bishop (1969) but radiologists will probably never encounter an unusual but urgent situation more than once. A recapitulation seems justified with the pre-



Fig 2 Survey film about 24 hours after the first rupture. 1) frontal view. Increased gas distension. Contrast meal passed through the small bowel to cecum and ascending colon.

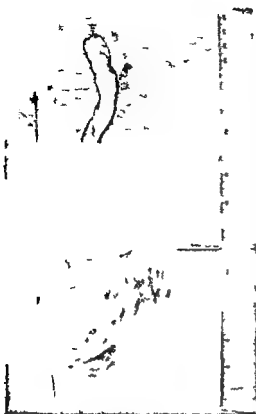


Fig. 3 Frontal view of the subtotally removed uterus. The uterus is partially expelled through a tear of the uterine wall.

classical example. The signs are: 1) The fetus is unexpectedly high in the abdominal cavity; 2) The fetus does not show the usual adjustment to the uterine wall; 3) The ordinary uterine shadow is absent; 4) Evidence of free fluid may be found in the peritoneal cavity.

In the present case points 1-3 were satisfied, but there was no evidence of free fluid in the peritoneal cavity. It seems plausible that the amniotic fluid had already been absorbed at the time of examination, as only a small amount of blood was present at the time. Even special procedures could not detect this.

The bowel distension in this case indicated some degree of ileus. A mechanical bowel obstruction was suspected and consequently the minor paralysis of the bowels may have been due to peritoneal irritation. Since signs of intestinal dysfunction obscured the general condition, the aforementioned four cardinal signs may be supplemented with a fifth: 5) Signs of paralytic intestinal distension.

Other diagnostic methods could have proven

conclusive if available. Firstly, diagnostic sonar should have demonstrated absence of the amniotic cavity, which must be practically pathognomonic as no amniotic fluid was demonstrated in the vagina. Furthermore, by sonar a reasonably precise estimation of fetal age can be made, which in this particular case, however, is of minor concern. Secondly, the Doppler effect could have shown that the fetus was alive. This would indicate an urgent laparotomy in case of a correct diagnosis.

As far as differential diagnosis is concerned, certain items were not paid enough attention to, particularly the change of fetal lie. In retrospect, it is apparent from the survey films that the fetus was lying outside the uterus, intermingled with more or less distended loops of large and small intestine.

It is true that, except in labor, the real nature of a complication like this will seldom, if ever, be diagnosed initially in cases where no traumatic manoeuvres are being used and no prior damage to the uterine wall is known of. Furthermore, in the event of the placenta covering the site of the future rupture, acute and probably violent symptoms will occur and be quite different from those of the present case. It must, however, be admitted that in this case, with the placenta left in situ for several days after the rupture, the diagnosis was unduly delayed. The initial suspicion of premature labor is understandable and was partly correct. Later, a concealed minor abruptio placentae was suspected. This too was partly correct. This diagnosis, however, should have been abandoned on the third day at the latest.

As far as the etiology of uterine rupture is concerned, the most common cause is the absence of traumatic manoeuvres in a post-caesarean scar. In this case, the only possible damage to the uterus is the curettage performed nine months earlier, following an early spontaneous abortion. This etiology has been suggested, e.g., by Åstedt et al. (1967). As the operative report throws no light on this possibility and as no myometrial fragments were found on microscopic examination, we would merely point out that by use of vacuum aspiration, the risk of perforating and damaging the uterine wall with an ovum forceps, a curette or a dilator will be avoided. And furthermore, in an emergency such as heavy bleeding in case of late or missed abortion, we think it wiser to empty the uterine cavity digitally by means of a small incision vaginally than to use brisk instrumental manoeuvres.



Fig 1 Abdominal surveyfilm, lateral view. High and transverse lie of the fetus surrounded by gas-distended loops of the large and small bowels

During the following two days the symptoms became more severe and in particular the colicky pains. The patient grew rather pale. She was still afebrile and had normal or slightly elevated blood pressure and a pulse rate of ~100/min. The abdomen had become distended and tympanic. Radiological examination of the abdomen beginning on the fourth day revealed a fetus lying transversely at the umbilical level and a severely gas-distended colon (Fig 1). Barium contrast enema failed to disclose low placed obstruction of the colon (Fig 2).

In view of the patient's state and the findings surgical intervention was considered necessary. The laparotomy revealed that the greatly distended sigmoid concealed the other abdominal organs. A small amount of blood was present in the cavity which in its upper part was taken up by the fetus with its head against the spleen. The infant was dark blue and had a low muscle tonus, the Apgar score being <3. The umbilical cord was immediately cut and adequate resuscitative steps taken. The infant soon recovered, grew pink and started to cry. Through a transverse tear of the uterine fundus the placenta was partially expelled (Fig 3). Due to the extensive damage to the wall a subtotal hysterectomy was performed.

Microscopically: the uterine wall at the site of the rupture showed signs of marked non-specific inflammation with degenerative changes of the myometrium. No further pathological changes were observed as far as the placenta and uterus were concerned.

As regards the child, the birth weight being 2600 g and the length 45 cm, its arterial blood pH was 6.8 initially. The acid-base data returned slowly to the normal range. So far its development has progressed normally. The post-operative course of the mother was uneventful.

DISCUSSION

The clinical symptoms and signs are explained by the following sequence of events: increasing uterine activity, the tensile part of the fundal wall eventually small rupture, the edges of which separate slowly. Between the 2nd membranes ruptured. Thereafter the fetus is expelled into the abdominal cavity, thus lie. The uterine activity must then considerably and the placenta remain a result of which the uteroplacental circulation kept adequate for nutrition of the fetus—and thereby paralytic ileus. At the time of laparotomy the uterine activity causing separation of the placenta was partially expelled through the tear (Fig 3). The utero-placental circulation rapidly bringing about an acute and asphyxia.

The radiological signs characteristic of rupture have been described by Parkinson (1958) and Bishop (1965). Radiologists will probably never encounter an unusual but urgent situation more than one recapitulation seems justified with the



Fig 2 Survey film about 74 hours after the first one. 1) frontal view. Increased gas distension of the colon. Barium contrast meal passed through the small bowel, cecum and ascending colon.

A CASE OF MASSIVE UNILATERAL OEDEMA OF THE OVARY SIMULATING TUMOUR

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Abstract This paper describes the first reported Scandinavian case of massive unilateral oedema of the ovary. It represents the tenth such case in the literature. The probable pathogenesis is partial torsion of the mes-ovarium. If suspected clinically, such ovaries might be conserved.

Massive oedema of the ovary is a newly recognized copathologic entity. The pathogenesis is the same as for haemorrhagic infarction from which massive oedema differs in the following respects: 1) impairment of venous and lymphatic drainage is not sufficient to cause necrosis; 2) the clinical picture is not hyperacute; 3) the condition is easily mistaken for tumour; 4) the possibility of conserving the ovary; and 5) the occurrence of stromal luteinization with ovarian dysfunction in the more longstanding cases. The treatment of the nine previously reported cases and the present case was total resection, although these ovaries should have been conserved.

CASE HISTORY

A 25-year-old previously healthy nullipara was admitted to hospital with a diagnosis of left-sided ovarian cyst. Menarche at age 13½. Menstrual cycle was regular. Five days before admission the patient complained of intermittent pain in the left iliac fossa. Physical examination revealed a normally developed 15-year-old girl without hirsutism. The gynaecological examination revealed a non-tender grapefruit-sized cystic mass in the lower left part of the abdomen. Hormonal studies were done. At laparotomy the left ovary was found to have been replaced by a somewhat tense tumour. The pedicle was twisted three times. The right ovary was slightly enlarged but was not biopsied. Left salpingo-hysterectomy was carried out. The postoperative course was uneventful.

Pathology

The left ovary measured 12½ × 7 × 6 cm and weighed 235 g. The capsule was intact. When cut the tissue was grey-white, moist, with scattered small hemorrhages and several cysts with a diameter up to 1 cm, containing clear fluid (Fig. 1).

Microscopic sections of the ovary showed oedema most pronouncedly in the medulla and internal cortex, whereas the tunica albuginea was only slightly involved. The lymph vessels, capillaries, veins and arteries were dilated, showing congestion. There was no stromal luteinization (Fig. 2).

COMMENT

Nine cases of massive unilateral oedema of the ovary (1, 2, 3, 4, 7) have previously been published. The condition presents clinically either in an acute-subacute form (7 cases) with intermittent abdominal pain lasting weeks to months, including the present case, or in a more chronic form (3 cases) without abdominal pain.

The most probable developmental factor is partial torsion or kinking of the mes-ovarium, compromising venous and lymphatic drainage but insufficient to cause necrosis (2, 4).

This concept is supported both by the microscopic picture and by the fact that torsion of the mes-ovarium was found at operation in four cases. In no case was there evidence of a previous pathologic process in the ovary which could predispose to torsion of the mes-ovarium.

In two of the previously published chronic cases, evidence of virilization and stromal luteinization was seen (2, 4). One case (3) showed slight stromal luteinization but no virilization. In all three cases, biopsy of the other ovary showed no pathological changes, thereby differentiating the condition from the Stein-Leventhal syndrome complicated by tor-



Fig 1 Oedematous tumour like left ovary

sion. Kalstone et al (2) mentioned that stromal luteinization could be a reaction to mechanical stretching of the stromal cells due to oedema analogous to the stromal luteinization and virilization which can be caused by infiltrative primary and metastatic carcinoma of the ovary even though the neoplastic cells themselves are non hormone producing (5, 6).

All cases were clinically mis-diagnosed ovarian neoplasms. The patients were 12 women 13–33 years of age. In this age-group ovary should if possible be conserved. As suggested by Kalstone et al (7) one should be suspicious of the diagnosis of massive oedema of ovary with the use of multiple frozen-section biopsies as well as attempting to secure the ovary in a position preventing torsion of the pedicle. In case of dysfunction one should perform a wedge resection to restore normal function of the ovary. In order to evaluate pathogenic factors one should also perform a wedge biopsy of the other ovary.

CONCLUSION

Massive unilateral oedema of the ovary caused by partial torsion of the mes ovarium is a rarely described cause of sub acute abdominal pain or irritation in younger fertile women.

The condition has in all cases led to total or partial resection although an attempt should be made to preserve these ovaries.



Fig 2 Microscopic section showing oedema of the ovary. Hematoxylin-eosin $\times 220$.

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ANNOUNCEMENTS

Secretariat of F I G O

Information received on the following congresses

VI International Congress of Cytology Tokyo Japan
May 2-5 1977

Themes relating to cytology

Information for information contact Dr A Meisels
Secretary General of the Congress 1050 Chemin Sainte
Foy Quebec PQ Canada

XI Acta Endocrinologica Congress Lausanne Switzer-
land June 19-23 1977

Themes relating to endocrinology

Information for information contact Professor J P
Felber Secretary General of the Congress Biochimie
Clinique Hopital Cantonal Universitaire 1011 Lausanne
Switzerland

*VI International Congress of Psychosomatic Obstetrics
and Gynecology* Rome Italy November 13-19 1977

Information for information contact Professor L Zich-
ella Department of OB/GYN Università degli Studi di
Roma Policlinico Umberto I 00161 Roma Italy

*An International Symposium on Genito-Urinary Tricho-
monosis* will be held in Paris 8-9 July 1977

For all information please write to Doctor A Fari-
S I T G U 5 Blvd de Strasbourg 75010 Paris France

The Fifth International Conference on Birth Control
will be held in the Queen Elizabeth Hotel V
Canada from 21-27 August 1977

For a complete program please apply to the Sec-
retary General Holland Organizing Centre 16 Lange Voor-
hout The Netherlands

The Fourth UICC Training Course in Cancer Research
arranged by the International Union Against Cancer
take place on 4-17 September 1977 in Budapest
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THE MYOMETRIAL RESPONSE TO INTRA UTERINE ADMINISTRATION OF PGF_{2α} AND PGE₂ IN DYSMENORRHEIC WOMEN

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Serial recordings of uterine contractility during all phases of the menstrual cycle were carried out in dysmenorrheic women and in two subjects during the stage of severe dysmenorrheic pain. The sensitivity and reactivity of the uterus to intra uterine administration of small doses of PGF_{2α} and PGE₂ was studied. A relative insensitivity of the uterus was found in the proliferative phase and around ovulation. The uterus was highly sensitive to the stimulatory effect of PGF_{2α} in the secretory phase and at menstruation but was not more sensitive than in normal women. The responding results with PGE₂ were rather variable in small doses did not induce any response or slight inhibition whereas high doses occasionally caused an increase in the secretory phase and during menstruation. The results indicate that PGF_{2α} may be a significant factor in eliciting uterine hypercontractility and dysmenorrheic pain. Whether the occasional inhibitory effect of PGE₂ is of any significant importance in the pathogenesis of dysmenorrhea cannot be stated.

Most studies on the etiology of dysmenorrhea have been focused on the role of the prostaglandins (1-6). Pickles initiated this concept by claiming that higher concentrations of PGF_{2α} occurred in the myometrium and menstrual fluid in women with dysmenorrhea than in those not suffering from menstrual pain on the basis of a bio-assay method. He suggested that the ratio PGF_{2α}/PGE₂ was of importance in eliciting menstrual cramps. Similar hypotheses have been made by other investigators using radioimmunoassay technique. Judging from the large variation in the reported concentrations it is evident that it is difficult to determine tissue concentrations adequately. Nevertheless it is a fact that severe menstrual pain can be completely eliminated in the majority of cases by the administration of prostaglandin synthetase inhibitors.

Martin & Bygdeman have studied the sensitivity of the myometrium to threshold doses of PGF_{2α} and PGE₂ administered into the uterine cavity in normal non pregnant women during various phases of the menstrual cycle (9-10). They found a stimulatory effect of small doses of PGF_{2α} and PGE₂ in the proliferative and secretory phases whereas the uterus was relatively insensitive both to PGF_{2α} and PGE₂ at ovulation. In contrast PGE₂ resulted in inhibition of uterine contractility during menstruation. The problem whether the myometrial sensitivity to PGF_{2α} and PGE₂ is different in dysmenorrheic subjects than in normal women has not been studied.

The following investigation was performed to analyse the sensitivity and reactivity of the uterus in women with primary dysmenorrhea to intra uterine administration of PGF_{2α} and PGE₂.

PATIENTS AND METHODS

Five women with consistent primary dysmenorrhea and regular menses volunteered for the study. Four subjects were nulliparous and one had dysmenorrhea even after two deliveries. All cases ovulated according to basal body temperature curves and determinations of progesterone in plasma during the secretory phase of the cycle. All volunteers had normal gynecological examination findings. A condom was recommended as a contraceptive agent during the course of the investigation and coitus was prohibited within 24 hours prior to each recording of uterine contractility. Reference to the time of recording in this paper is based upon the number of days before (-) or after (+) the estimated date of ovulation.

Threshold doses of PGF_{2α} were administered into the uterine cavity in 3 subjects on four occasions during the cycle: in the proliferative phase around ovulation, in the secretory phase and at menstruation. This part of the study comprised 12 recordings.

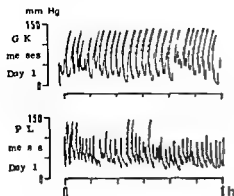


Fig 1 Uterine contractility pattern in 2 patients recorded on the first day of menstrual bleeding and during the period of maximum dysmenorrheic pain. Note the high level of uterine tonus and amplitude of contractions

Local administration of PGE_2 was made in 4 women, 2 of whom had earlier taken part in the $\text{PGF}_{2\alpha}$ treated group. In these 2 women recordings were carried out in the secretory phase and at menstruation. In the other 2 subjects recordings were made on four occasions in different phases of the cycle. Consequently 12 recordings were also made in the PGE_2 group.

Two other women were recorded during menstruation in the presence of severe dysmenorrheic pain. For ethical reasons prostaglandin was not administered in these cases.

Diluted solutions of $\text{PGF}_{2\alpha}$ and PGE_2 were prepared from concentrated stocks before each recording session. The activity of the stock solution of PGE_2 was tested by ultraviolet absorption following purification on thin layer chromatography and conversion to PGB with alkali before the investigation and after completion of the series of

There was no evidence of decreased activity.

Vials containing the stock solutions were kept deep until used. The volume of fluid instilled into the cavity varied between 0.1 and 1.4 ml. Single doses of 1–10 μg $\text{PGF}_{2\alpha}$ were given and the corresponding doses for PGE_2 were in the range of 1–40 μg . When high doses of PGE_2 were administered the concentrated stock solution was used.

Uterine contractility was recorded by the micro balloon technique. The size of the uterine cavity was determined by a probe and the tip of the balloon (capacity 0.1 ml, diameter 2 mm, length 20 mm) placed approximately one cm below the fundus. The catheter was connected to a Statham pressure transducer and a Grass polygraph. A thin polyethylene catheter for instillation of the prostaglandin compounds into the uterine cavity had previously been tied in the micro balloon catheter. The catheters were kept in position by wedging three gauze swabs into the vagina and around the catheters. The basic contractility pattern was recorded for a period of at least 30 minutes before the first injection of $\text{PGF}_{2\alpha}$ or PGE_2 .

A stimulatory or inhibitory response is difficult to measure in the individual case. However 19 of the 24 original recordings are illustrated as evidence of the conclusions.

RESULTS

The uterine contractility pattern during the proliferative and secretory phases of the cycle was similar to that observed in subjects who had no dysmenorrhea. During the proliferative phase the contractions were characterized by a low amplitude and comparatively high frequency. Around ovulation there was an increase in frequency and a decrease in amplitude. During the secretory phase there was a gradual increase in amplitude and a decrease in frequency. Premenstrually developing contractions were more complex or labour like contractions. In the first day of menstrual bleeding and in the presence of dysmenorrheic pain the contractility pattern was characterized by marked hypertonus and high amplitude contractions or frequent complex contractions without relaxation between them (Fig 1).

Intrauterine administration of PGF_2

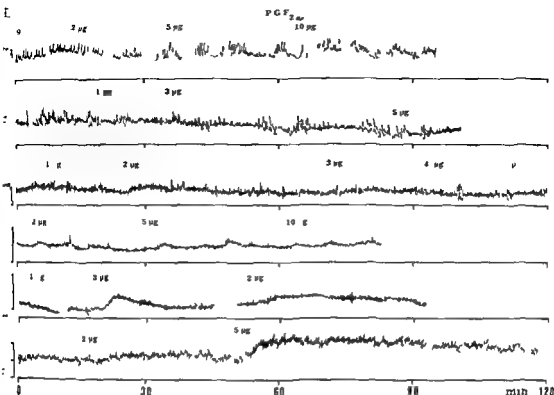
In 3 volunteers studied in the proliferative phase there was no significant response to doses of 10 μg $\text{PGF}_{2\alpha}$. Of the 3 volunteers studied around ovulation one case did not react to doses up to 10 μg $\text{PGF}_{2\alpha}$ while the 2 other subjects responded to stimulation upon instillation of 1 and 5 μg respectively (Fig 2).

A marked increase in sensitivity to $\text{PGF}_{2\alpha}$ occurred during the secretory phase (Fig 3). Ten different recording sessions in 5 volunteers from day +7 to +14 (21st–28th day of the cycle). Four cases responded with stimulation to 1 μg $\text{PGF}_{2\alpha}$, one volunteer to 5 μg $\text{PGF}_{2\alpha}$. The latter was recorded on day +10 (24th day of the cycle) and by a rise in tonus of 25 mmHg and an increase in amplitude of 30–40 mmHg (A.T. Fig 3). The subject experienced severe menstrual pain and on this rather small dose of $\text{PGF}_{2\alpha}$. The other volunteers obtained moderate menstrual pain. In one in addition nausea following 5 μg $\text{PGF}_{2\alpha}$. A similar response was also obtained in one case on the 4th day of her menstrual bleeding (Fig 4).

Intrauterine administration of PGE_2

Administration of PGE_2 resulted in a more complex response than that of $\text{PGF}_{2\alpha}$. Uterine sensitivity was variable around ovulation. One case reacted to 2 μg PGE_2 , another case to 10 μg stimulation and a third subject was insensitive to 30 μg when tested on day –3 and on day +7.

In the mid secretory phase there was a decrease in uterine tonus in one case following a large



Uterine sensitivity to local administration of PGE_2 in the proliferative phase and at ovulation of normoaltr women

(70 µg) whereas lower doses (2 and 5 µg) had doubtful effects in 2 other volunteers (Fig. 4). Cases recorded in the late secretory phase did not respond to stimulation following 2 µg and 10 µg of PGE_2 (Fig. 5). No recordings were obtained during menstruation at a time when the period of dysmenorrhea had already passed. These curves illustrate that a small dose of PGE_2 (2 µg) stimulated the activity whereas a large dose (37 µg) significantly decreased the amplitude (Fig. 5). All patients experienced little or no dysmenorrheic pain as a result of these injections.

DISCUSSION

The micro-balloon technique for recording uterine activity has been shown to give identical recordings as those obtained with the open-end catheter method provided that the size of the balloon is small (4). Under our experimental conditions the former method turned out to be more simple and reliable than the latter. In an earlier study carried

out in this department by Martin & Bygdeman control injections of physiological saline solution into the uterine cavity did not result in any change in uterine contractility (9). Therefore it was considered unnecessary to repeat this type of control study as the technique of instillation and recording was the same in these two investigations. The instilled volumes varied between 0.1 and 1.4 ml. It is impossible to evaluate the significance of the volume factor upon the results. However the undiluted stock solutions were administered directly when large doses were given.

The basic contractility pattern in the dysmenorrheic subjects was approximately the same as that in normal women during the cycle. The only difference seemed to be the period of dysmenorrheic pain at menstruation when the uterine tone and amplitude and frequency of contractions were consistently high. This concept has been controversial for many years but evidence is accumulating in support of uterine contractility and decreased uterine blood flow as important factors in the mechanism of dysmenorrhea (5). It is also a fact

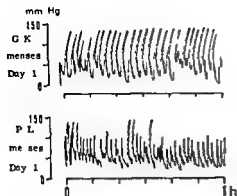


Fig 1 Uterine contractility pattern in 2 patients recorded on the first day of menstrual bleeding and during the period of maximum dysmenorrheic pain. Note the high level of uterine tonus and amplitude of contractions.

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A stimulatory or inhibitory response is difficult to measure in the individual case. However, 10 of the 24 original recordings are illustrated as evidence of the conclusions.

RESULTS

The uterine contractility pattern during the proliferative and secretory phases of the cycle was similar to that observed in subjects who had no menses. During the proliferative phase the contractions were characterized by a low amplitude and comparatively high frequency. Around ovulation there was an increase in frequency and a decrease in amplitude. During the secretory phase there was a gradual increase in amplitude and a decrease in frequency. Premenstrually developed more complex or labour-like contractions on the first day of menstrual bleeding and in the presence of dysmenorrheic pain the contractility was characterized by marked hypertonic, high amplitude contractions or frequent complex contractions without relaxation between them (Fig 1).

Intrauterine administration of $\text{PGF}_{2\alpha}$

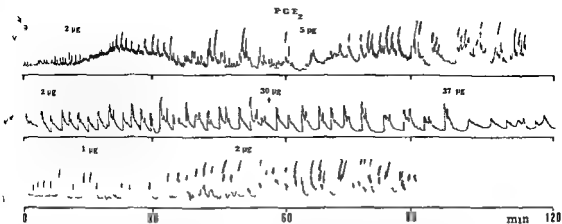
In 3 volunteers studied in the proliferative phase there was no significant response to doses of 1–10 μg $\text{PGF}_{2\alpha}$. Of the 3 volunteers studied around ovulation one case did not react to doses up to 10 μg $\text{PGF}_{2\alpha}$ while the 2 other subjects responded to stimulation upon instillation of 2 and 3 μg respectively (Fig 2).

A marked increase in sensitivity to $\text{PGF}_{2\alpha}$ occurred during the secretory phase (Fig 3). In 5 different recording sessions in 5 volunteers from day +7 to +14 (21st–28th day of the cycle) 4 cases responded with stimulation to 1–5 μg $\text{PGF}_{2\alpha}$. One volunteer to 5 μg $\text{PGF}_{2\alpha}$. The latter case was recorded on day +10 (24th day of the cycle) and by a rise in tonus of 25 mmHg and an increase in amplitude of 30–40 mmHg (A-T Fig 3). The subject experienced severe menstrual pain and on this rather small dose of $\text{PGF}_{2\alpha}$. The other 4 volunteers obtained moderate menstrual pain, one in addition nausea following 5 μg $\text{PGF}_{2\alpha}$. A similar response was also obtained in one volunteer on the 4th day of her menstrual bleeding (Fig 4).

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In the mid secretory phase there was a large increase in uterine tonus in one case following a large



Uterine response to local administration of PGE_2 in secretory phase and III menstruation. Both the during menstruation were recorded when the

period of dysmenorrheic pain had passed. Note the stimulatory effect of small doses (G, L) and the inhibitory response to a large dose (K, H).

a significant relaxation of uterine contractility following administration of prostaglandin synthetase inhibitors or beta stimulating drugs is associated by a marked increase in uterine blood flow and disappearance of dysmenorrheic pain.

Run & Bygdeman found that the uterus of normal women was highly sensitive to the intra-uterine administration of $PGF_{2\alpha}$ both during the proliferative and secretory phases of the cycle in that a stimulatory response was obtained with doses in the range of 1–3 µg. A decreased sensitivity was noted in the periovulatory period when doses ranging from 4 and 8 µg did not result in any response. These results differ to some extent from those of the present study. The uterus did not react to doses of 10 µg in the proliferative phase whereas an increase in sensitivity occurred in the secretory phase where all cases responded by stimulation of uterine contractility following administration of 1 µg $PGF_{2\alpha}$. This means that a significant increase in sensitivity does not occur until the mid or late secretory phase and that uterine insensitivity is a particular feature for the periovulatory period and the proliferative phase.

Whether the difference in results between the two studies reflects a difference between normal women and dysmenorrheic subjects cannot be answered on the basis of available data. The uterine response is highly variable under the prevailing experimental conditions and the number of experiments in both Run & Bygdeman's study and the present investigation is limited. There is at any rate an agree-

ment between the two studies that the uterus is sensitive to small doses during the secretory phase and it may be of some interest that uterine sensitivity to $PGF_{2\alpha}$ of dysmenorrheic women in the premenstrual period is not higher than that of normal women.

The period of dysmenorrheic pain during menstruation is of course the most relevant time for measuring uterine sensitivity to $PGF_{2\alpha}$ considering the aim of the present investigation. For ethical reasons it was not possible to administer $PGF_{2\alpha}$ in the presence of severe pain. The presence of an unknown volume of menstrual blood in the cavity resulting in dilution of the injected dose and containing an unknown amount of endogenous prostaglandins may also create uncertainty about the actual effective dose. However, one recording on the 4th day of menstruation when the stage of uterine cramping and pain was passed illustrates at least that the uterus at menstrual bleeding has a similar sensitivity to that during the secretory phase.

In dysmenorrheic women, small doses of intra-uterine PGE_2 (2–5 µg) either stimulated the contractility or had no effect at all around ovulation in the secretory phase and at menstruation. Large doses (20–40 µg) on the other hand occasionally had a transient inhibitory effect. The latter response was observed both in the secretory phase and during menstruation which is in agreement with results obtained in *in vitro* experiments with human myometrial strips (3). Approximately the same results were obtained in non-dysmenorrheic women.

in *in vivo* studies by Topozada et al and by Martin & Bygdeman (14, 10). The former authors found that the inhibitory response could be elicited in postmenopausal women and in one case during the secretory phase of the cycle but only following very large doses (200–300 µg). Moreover, the ingestion of contraceptive pills caused a reduced uterine sensitivity to prostaglandin (15). This is of interest considering the fact that oral contraceptives eliminate or reduce dysmenorrheic pain. Martin & Bygdeman observed the inhibitory effect of PGE_2 only during menstruation.

These results are in contrast to the finding that intravenous administration always results in stimulation irrespective of dose and phase of cycle (11). Another difference between local and systemic administration of prostaglandins relates to the threshold dose levels. Ten to twenty times higher doses are needed if the compound is given as a single intravenous injection than if the substance is injected directly into the uterine cavity. This may be due to the rapid inactivation process of prostaglandins in the circulation and the lack of inactivating enzymes within the uterus (7).

The relative significance of PGE_2 in the pathogenesis of dysmenorrhea is in this context still obscure since the compound apparently both increases and decreases uterine contractility. However, judging from the present results it seems probable that $\text{PGF}_{2\alpha}$ represents a key substance eliciting uterine hypercontractility and dysmenorrheic pain. It also appears that the uterus of dysmenorrheic subjects has a similar sensitivity to $\text{PGF}_{2\alpha}$ and PGE_2 as in normal women. If this conclusion is correct and valid as well during menstruation it would mean that dysmenorrheic pain should rather result from an increased intra-uterine formation of $\text{PGF}_{2\alpha}$ than be the expression of an increased uterine sensitivity to the compound.

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THE BEHAVIOUR OF MICROSOMAL MONO ELECTRON CARRIERS IN ADULT AND FETAL LIVER AND IN PLACENTA THROUGHOUT PREGNANCY

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Abstract The activities and contents of microsomal mono-electron carriers (NADPH cytochrome c reductase, NADH-cytochrome b_5 reductase, cytochrome P-450 and cytochrome b_5) in the liver of 160 pregnant rabbits and their response to phenobarbital (PB) administration were studied to be different to that of non pregnant animals and vary depending on the period of gestation. In fetal liver and in placenta the activities and contents of mixed function oxidase system enzymes are very low and increase at the mid gestation up to the term of pregnancy. PB has either no effect or decreases the enzyme levels. The variations of enzyme content and activity in maternal liver show the different response to PB are related to the hormonal modifications of pregnancy. The behaviour of mono-electron carriers in fetal liver can be related to the process of maturation of this organ. The effect of PB can be due to the interference of the drug with steroid metabolism. The behaviour of mixed function oxidase system enzymes in placenta can be related to the process of maturation and ageing of the organ.

It is well known that the mixed function oxidase system is of great importance in the metabolism of drugs and hormones etc (16-53). Microsomal mixed function oxidase system enzymes are present in very low quantities in embryonic tissues (fetal liver) and in placenta. After birth they increase progressively until they reach adult levels in a specific period of time depending on the animal species considered (7, 8, 10, 11, 17, 20, 21, 27, 35, 37, 40, 44, 47, 51, 56, 57, 60, 66).

The aim of this work is the following: (a) to study the quantitative relations and the behaviour of the microsomal mono-electron carriers (of cytochrome P-450, of cytochrome b_5 , of NADPH cytochrome c reductase and of NADH cytochrome b_5 reductase) in maternal and in fetal liver and in placenta of rabbits; (b) to study the microsomal mono-electron

carriers after administration of an enzymatic inducer (18) phenobarbital (PB). The results obtained by others on this subject do not all agree (1, 3, 12, 36, 52, 54, 55, 62-64).

Our results show that (a) in rabbit's liver gestation modifies the levels of mixed function oxidase system enzymes; variations are different in each one of the four enzymes; response to administration of PB is different to that of the non pregnant animal and varies depending on the period of gestation; (b) in fetal liver and in placenta the mixed function oxidase system enzymes are very low at mid gestation and increase at term. PB treatment is ineffective or acts as an inhibiting factor on the mixed function oxidase system enzymes.

The mechanism responsible for these modifications are discussed.

MATERIALS AND METHODS

17 white New Zealand female rabbits weighing 2.5-3.5 kg and 160 pregnant rabbits weighing 2.5-4 kg were used. A treatment was effected with a subcutaneous injection of 30 mg of sodium phenobarbital (PB) per kg/body weight each day for three days before the animals were killed. This was done by means of a blow on the head followed by cervical dislocation, always at the same time of the day (9 a.m.). Microsomal drug metabolizing enzymes were determined on pooled tissue from all the animals. On the 12th day of gestation determinations on fetal liver were not carried out as material was insufficient. Livers and placentae which were rapidly removed after death, were poured into 0.15 M KCl containing 50 mM Tris HCl buffer pH 7.5 at 0-4°C.

The microsomal fraction obtained according to Omura and Sato (39) was suspended in the above mentioned medium yielding a protein concentration of about 15 mg/ml. For fetal liver and placenta in the earlier stages of gestation since there was very little tissue it was not

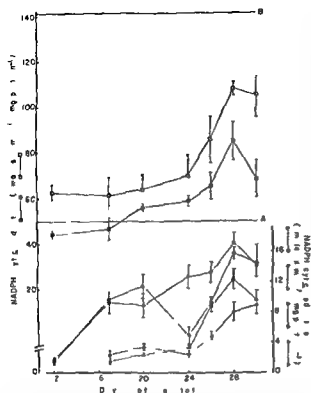


Fig 1 Kinetics of microsomal NADPH cytochrome c reductase from various organs in rabbits at different pregnancy ages ■—■ maternal liver ▲—▲ fetal liver ●—● placenta □—□ maternal liver after 3 days treatment with PB (30 mg/kg b.wt s.c.) △—△ fetal liver after 3 days treatment with PB (30 mg/kg b.wt s.c.) ○—○ placenta after 3 days treatment with PB (30 mg/kg b.wt s.c.) Line A represents the level of microsomal NADH cytochrome c reductase from liver of non pregnant control animals line B from non pregnant PB treated animals. The enzyme activity has been measured as described in Materials and Methods

possible to obtain the same concentrations hence concentrations close to 15 mg/ml were used. Protein were determined by the biuret method (32). NADPH cytochrome c reductase was measured following cytochrome c reduction at 546 nm in the presence of NADPH (65). NADH cytochrome b_5 reductase was measured following NADH oxidation at 340 nm in the presence of potassium ferricyanide as electron acceptor (59). Cytochrome P-450 and cytochrome b_5 were determined by a dual wavelength split beam Aminco Chance spectrophotometer. The amount of cytochrome b_5 was calculated on NADH reduced samples using $\Delta\epsilon(424-409 \text{ nm}) = 165 \text{ cm}^2 \times \text{mM}^{-1}$ (14). Cytochrome P-450 was measured on CO treated-dithionite reduced-microsomes using $\Delta\epsilon(450-490 \text{ nm}) = 91 \text{ cm}^2 \times \text{mM}^{-1}$ (29).

RESULTS

NADPH cytochrome c reductase

In maternal liver the enzymatic activity of NADPH cytochrome c reductase increases progressively

from the 17th to the 28th day of gestation and increases slightly after PB treatment. On the 28th day, NADPH cytochrome c reductase is reduced after the PB induction maintained (Fig 1).

Enzymatic activity in fetal liver is very low until the 24th day of gestation and is not modified by PB. After the 24th day it increases progressively and is slightly inhibited by PB. On the 30th day it has decreased and is not modified by the treatment (Fig 1).

In placenta activity of NADPH-cytochrome c reductase is very low on the 12th day. Afterward it increases and reaches its maximum value on the 28th day. Initially the PB treatment is ineffective and is inhibiting in the second stage of gestation (Fig 1).

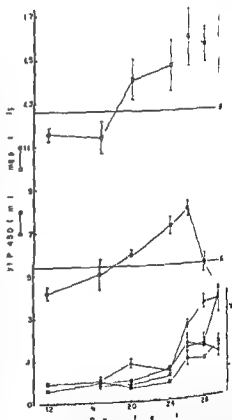


Fig 2 Kinetics of microsomal cytochrome P-450 in various organs in rabbits at different pregnancy ages ■—■ maternal liver ▲—▲ fetal liver ●—● placenta □—□ maternal liver after 3 days treatment with PB (30 mg/kg b.wt s.c.) △—△ fetal liver after 3 days treatment with PB (30 mg/kg b.wt s.c.) ○—○ placenta after 3 days treatment with PB (30 mg/kg b.wt s.c.) Line A represents the level of microsomal cytochrome P-450 from liver of non pregnant control animals line B from non pregnant PB treated animals. The enzyme activity has been measured as described in Materials and Methods

cytochrome P-450

Content of cytochrome P-450 in *maternal liver* increases progressively up to the 26th day. Afterwards it decreases to initial values. PB treatment on the 26th day of gestation increases the content of cytochrome P-450 100% and by about three times as much on the 30th day when basic values are very low (Fig. 2).

The content of cytochrome P-450 in *fetal liver* is low up to the 24th day and increases rapidly in the last days of gestation. PB determines a marked increase of enzyme content on the 26th and the 30th day of gestation; it is ineffective on the 30th day (Fig. 2). The content of cytochrome in *placenta*, *fetal liver* is very low up to the 24th day of

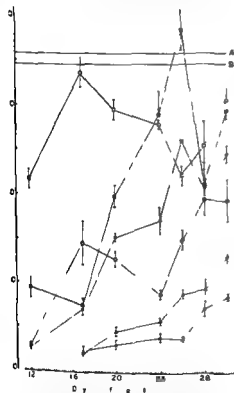


Fig. 3 Kinetics of microsomal NADH-cytochrome b_5 reductase from various organs in rabbits at different pregnancy ages: ■—■ maternal liver; ▲—▲ fetal liver; □—□ maternal liver after 3 days treatment with PB (30 mg/kg b.wt. s.c.); ▲—▲ fetal liver after 3 days treatment with PB (30 mg/kg b.wt. s.c.). Line A represents the level of microsomal NADH-cytochrome b_5 reductase from liver of non-pregnant control animals; line B from non-pregnant PB-treated animals. The enzyme activity has been measured as described in Materials and Methods.

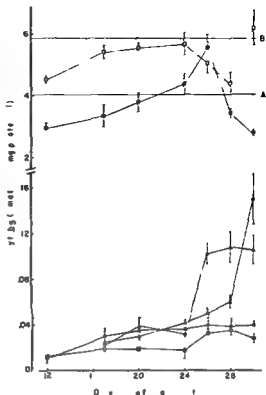


Fig. 4 Kinetics of microsomal cytochrome b_5 from various organs in rabbits at different pregnancy ages: ■—■ maternal liver; ▲—▲ fetal liver; □—□ maternal liver after 3 days treatment with PB (30 mg/kg b.wt. s.c.); ▲—▲ fetal liver after 3 days treatment with PB (30 mg/kg b.wt. s.c.). Line A represents the level of microsomal cytochrome b_5 from liver of non-pregnant control animals; line B from non-pregnant PB-treated animals. The enzyme activity has been measured as described in Materials and Methods.

gestation. Later cytochrome P-450 increases. PB treatment determines slight inhibitions from the 24th to the 28th day (Fig. 2).

NADH cytochrome b_5 reductase

NADH-cytochrome b_5 reductase activity in *maternal liver* is by far inferior to that of the controls up to the 17th day of gestation. It has a high and rapid increase up to the 26th day and decreases in the following days. PB treatment, which is ineffective in non-pregnant animals, causes an increase of NADH cytochrome b_5 reductase between the 12th and the 20th day; in the days that follow it is either ineffective or has an inhibiting effect on the activity of the enzyme (Fig. 3).

In *fetal liver* NADH-cytochrome b_5 reductase increases progressively starting from the 17th day.

The enzyme activity is inhibited by PB treatment (Fig. 3)

In *placenta* the enzyme's behaviour corresponds to the one observed in maternal liver. The effects of PB treatment are variable depending on the period of gestation (Fig. 3)

Cytochrome b_5

Cytochrome b_5 in *maternal liver* is initially present in concentrations lower than those of the controls and increases up to the 26th day. It goes back to the initial values after the 26th day. PB treatment determines a variable increase of the cytochrome b_5 concentrations depending on the day considered (with the exception of the 26th day when this treatment is ineffective) (Fig. 4)

In *fetal liver* the content of the enzyme is very low up to the 24th day; it increases considerably and rapidly from the 24th to the 26th day. PB increases the concentration of the enzyme on the 30th day. During the other days it is either ineffective or decreases the content of the enzyme (Fig. 4)

In *placenta* there is a slight increase during the first few days (12th and 17th) after which these values remain constant. After PB treatment the content of the enzyme decreases (Fig. 4)

DISCUSSION

Maternal liver Numerous observations show that in the course of gestation there are modifications of metabolism of drugs (6, 13, 19, 28, 48) of the mixed function oxidase system enzymes content (19, 36, 55) and of the response to treatment with inducers (36, 55). Furthermore it has been observed that in male rats the metabolism of drugs *in vivo* decreases after estradiol treatment (50) and that oral treatment with combinations of estrogen and progesterin slows down the metabolism of antipyrine (38). These observations suggest that the effects of pregnancy on the metabolism of drugs may depend on hormonal variations. Our results show that during pregnancy the levels of microsomal mono-electron carriers in maternal livers are generally decreased. Variations are in relation to the stage of gestation. PB in a pregnant animal does not produce the same effects as in a non pregnant animal. During pregnancy PB scarcely induces the NADPH cytochrome c reductase; it clearly induces—and sometimes even to a higher degree than that of controls—cytochrome P-450; it considerably in-

duces NADH cytochrome b_5 reductase; it does not modify this enzyme in controls. The effects on cytochrome b_5 are different according to the period of gestation. The scarce induction of NADPH cytochrome c reductase—rate limiting enzyme of drugs oxidation (9, 15, 31)—by PB therefore explains the reduced metabolism of drugs during gestation. The slight induction of NADPH cytochrome c reductase by PB has not been observed in female non pregnant rabbits; in male rabbits treated with HCG (47, 48). In conclusion we can assume from previous observations (41) and from this work that the variations of mixed function oxidase system enzyme levels determine the different response of maternal liver to treatment with PB may depend on hormonal modifications.

Fetal liver Observations on the levels and activities of drug metabolizing enzymes in fetals are numerous and results do not always agree (3, 33, 46, 52, 62). Our observations demonstrate that the levels of drug metabolizing enzymes during gestation are very low and increase progressively during gestation. Some authors have noted that blood progesterone levels decrease starting from mid pregnancy (4, 22, 34) and that the hormone has an inhibiting effect on the mixed function oxidase system enzymes (5, 23–26, 58, 61). These observations could indicate that progesterone can be responsible for the behaviour of drug metabolizing enzymes during pregnancy. As far as the effects of PB are concerned it has been noted that PB generally has an inhibiting effect on enzyme activity which varies according to the stage of pregnancy. Since PB interferes with the metabolism of steroids (30) it is probable that the inhibiting effects of PB may be attributed to hormonal variations. It can therefore be concluded as probable that the different levels of the drug metabolizing enzymes in fetal liver and their variations in the course of pregnancy may be related, as in the case of maternal liver, to hormonal variations. Taking into due consideration, however, the problems pertaining to the metabolism of the organ.

Placenta It has been demonstrated that the capacity of placenta to metabolize drugs varies according to the stage of gestation (7, 10, 12, 39, 46) and that for some drugs it decreases with the progress of pregnancy (2, 10, 33). The results of our experiments show that the mixed function oxidase system enzymes are originally at very low levels. From the 12th day the NADPH cytochrome

se and NADH cytochrome b_5 reductase progressively increase towards the term of gestation after having reached the highest point of concentration begin to diminish. This decrease becomes more intense if pregnancy is prolonged and term (45). Similar behaviour has been noted in human placenta in the course of pregnancy at and beyond term (44). Cytochrome P-450 and cytochrome b_5 are at very low levels until the 24th week then they slowly increase towards term. This behaviour of mixed function oxidase system enzymes does not allow us to draw definite conclusions. However the behaviour observed is of very initial values, the increase that follows and the decrease at term are probably related to the stage of maturation and age of the placenta.

ACKNOWLEDGEMENTS

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EVALUATION OF FETAL MATURITY BY AMNIOTIC FLUID CREATININE CONCENTRATIONS AND LECITHIN/SPHINGOMYELIN RATIO

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Rikshospitalet Oslo Norway

act In a material of 144 samples of amniotic fluid the last trimester creatinine concentration lecithin/sphingomyelin (L/S) ratio and their correlation have examined. Amniotic fluid creatinine concentration increased gradually through the last trimester up to 37 weeks (273-279 days p.m.) with median values of 2.00 mg/100 ml at 37 weeks and 2.5 mg/100 ml at 39 weeks. The great range makes a f.c. of doubtful value for assessment of gestational age in individual samples. The relation between a.f.c. and L/S ratio was highly significant (Kendall's $T=0.56$). Even so lung maturity could be predicted from a.f.c. A low L/S ratio with ≥ 2 mg/100 ml was found in 8-9% while a.f.c. ≥ 9 mg/100 ml was combined with L/S ratios ≥ 2 in a number of cases. A.f.c. < 1 mg/100 ml was invariably correlated with low L/S ratios.

The purpose of the present study has been to evaluate a.f.c. in assessment of gestational age and to examine the correlation between a.f.c. and the L/S ratio. A.f.c. estimation is available in most laboratories but many hospitals have no access to spholipid analysis. A great number of studies have shown the relation between L/S ratio and the respiratory function in the newborn. Instead of L/S ratio in evaluation of lung maturity a.f.c. could be used if a good correlation existed among these two parameters.

MATERIAL AND METHODS

Amniotic fluid creatinine and L/S ratio have been examined in 144 samples of amniotic fluid from 97 pregnant women with known gestational age delivered in the Department of Obstetrics and Gynaecology, Rikshospitalet, from September 1974 to December 1975. 132 of the samples were taken in the last trimester and 12 samples between

26-28 weeks of gestation. Amniotic fluid was collected by transabdominal amniocentesis or at caesarean section. In 14 cases a transvaginal puncture was performed during spontaneous delivery. Samples contaminated by blood or meconium were discarded. The number of patients and samples in the different groups of patients are presented in Table I. Among the patients with normal pregnancies 13 were delivered by caesarean section because of mechanical disproportion.

The a.f.c. was analyzed by Autotechnicon SM 12/60 method (modified Jaffe's reaction with alkaline picrate) and L/S ratio determinations were performed by a modified Gluck's method as previously published (15).

The results of a.f.c. and L/S ratio determinations were correlated to gestational age and to each other and the correlation between the two parameters was statistically evaluated.

In samples taken within one week prior to delivery the results were related to the clinical outcome with regard to respiratory function and the a.f.c. was compared with the weight of the fetus. A growth retarded child is defined as a child ≤ 2.5 percentile according to Bjerkedal et al. (2). The assessment of the respiratory distress syndrome (RDS) has been previously published (9). Typical symptoms lasting for less than 24 hours are referred to as transitory RDS.

Table I Groups of patients included in the material

Groups of pats	No of pats	No of samples
Rhesus immunized	38	80
Diabetes mellitus (White C D F)	74	26
Preeclampsia and/or growth retardation	8	11
Normal pregnancies	27	27
Total	97	144

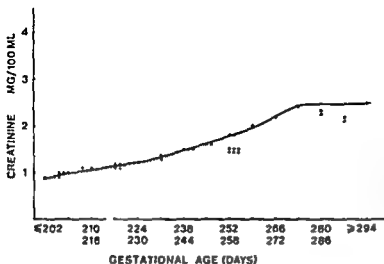


Fig 1 Creatinine concentrations in samples of amniotic fluid (gestational age) ● One median values

RESULTS

The a f c increased gradually through the last trimester up to 39 weeks (273–279 days p m). As seen in Fig 1 the increase in the median values was nearly linear with levels of 2 mg/100 ml at 37 weeks gestation and 2.5 mg/100 ml at 39 weeks. Prior to 35 weeks gestation values of ≥ 2 mg/100 ml seem to be exceptional and occurred in our series only in one patient with preeclampsia and a growth retarded fetus. After 35 weeks there is a great range in the results obtained and a f c < 2 mg/100 ml occurred occasionally even after 40 weeks.

The different categories of patients are not valid for comparison due to different numbers and different gestational age. However the a f c from the 40 mothers seem to be distributed as for immunized while the few preeclamptic patients (8 patients) have values in the upper part of the range. Maternal serum creatinine was ≤ 0.9 mg in 4 of these patients and was not examined in the re-

maining 4 mothers with mild preeclampsia was not of a degree likely to have any effect on renal function. There is no correlation between a f c and the weight of the fetus (Fig 2).

The L/S ratios demonstrate the normal variation according to gestational age (Fig 3) and the median values reach the considered critical value of about 35 weeks. However there are great individual variations. With a gestational age of 34 weeks the L/S ratio was 1.9 in one sample and 2.7 remaining 27. On the other hand a L/S ratio was not found in the 49 specimens taken prior to 34 weeks. No special trend was found for L/S ratios among the different categories of patients.

There is a positive correlation between a f c and L/S ratio. The numeric variation in L/S ratio is much greater than in a f c and in Fig 4 the correlation is illustrated on a semilogarithmic scale. Kendall's correlation coefficient between a f c and L/S ratio was calculated to 0.36 which is highly

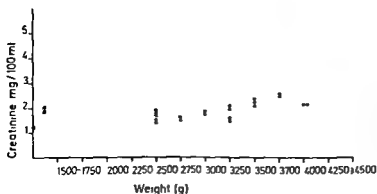


Fig 2 Creatinine concentrations in samples of amniotic fluid obtained at one week of delivery related to the weight of the newborn ● One sample from a fetus with no growth retardation ○ One sample from a growth retarded child (percentile—see text)

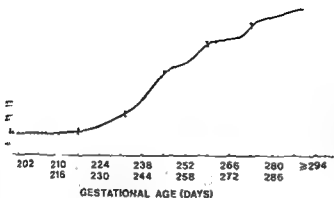


Fig 3 L/S ratio in 144 samples of amniotic fluid related to gestational age ● One sample of amniotic fluid — median values

icant ($P < 0.0005$) A f c levels ≥ 2 mg/100 ml only associated with L/S ratios below 2 in 6 cases only 4 of these really low (< 1.8) L/S ratios were however associated with a f c s in the range 1.1–1.9 in 27 samples A f c mg/100 ml were invariably associated with low ratios

40 cases the samples were obtained at delivery in a further 20 within one week prior to delivery Six of the 60 children developed RDS Two children from diabetic mothers who were only affected by RDS had L/S ratio 2.1 and 2.6 with a f c > 2 mg/100 ml The remaining 4 had ratios of 0.6 0.8 1.1 and 1.2 with corresponding f c values 1.1 1.8 1.6 and 1.5 (Fig 5) No child died from RDS

DISCUSSION

A consistent rise in a f c during pregnancy is documented by many authors (1 3 5 7 8 12 13 14 16) Values of ≥ 2 mg/100 ml are likely to be obtained prior to 35 weeks and indicate that the gestational age is probably 37 weeks or more but the great range makes a f c determination of limited value in individual samples a comment made in several papers (6 11) Values < 2 mg/100 ml occurred even at a gestational age of 40 weeks Roopnanesingh (14) and Weiss et al (16) found a positive correlation between birthweight and a f c—the latter up to a fetal weight of 2500 g In this study we found no correlation with the fetal weight Only 9 of the 60 children referred to were

delivered prior to the 36 weeks Premature infants generally have lower birthweights and a f c values but beyond that there is obviously no correlation between a f c and the weight of the fetus This gives support to the theory that a f c reflects the maturation of renal function in the fetus rather than its muscle mass and purine metabolism The high a f c values in patients with preeclampsia as associated with growth retarded fetuses are noteworthy and may be a result of reduced placental exchange and/or oligohydramnios Cassidy et al (3) Roopnanesingh (14) Bertram et al (1) and Lofstrand et al (10) have also stated that a f c is ele

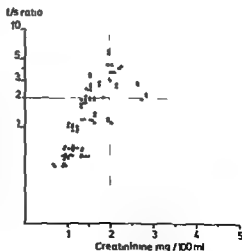


Fig 4 The correlation between L/S ratio and the creatinine concentrations in 144 samples of amniotic fluid illustrated on a semilogarithmic scale ● One sample of amniotic fluid

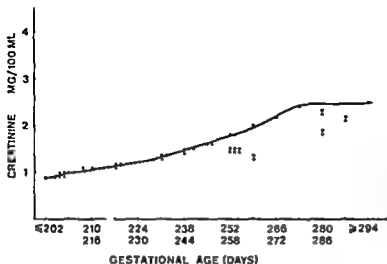


Fig 1 Creatinine concentration in samples of amniotic fluid related to gestational age. ● One sample of amniotic fluid. — median values

RESULTS

The α fc increased gradually through the last trimester up to 39 weeks (273–279 days p m). As seen in Fig 1 the increase in the median values was nearly linear with levels of 2 mg/100 ml at 37 weeks gestation and 2.5 mg/100 ml at 39 weeks. Prior to 35 weeks gestation values of ≥ 2 mg/100 ml seem to be exceptional and occurred in our series only in one patient with preeclampsia and a growth retarded fetus. After 35 weeks there is a great range in the results obtained and a α fc < 2 mg/100 ml occurred occasionally even after 40 weeks.

The different categories of patients are not valid for comparison due to different numbers and different gestational age. However the α fc from the diabetic mothers seem to be distributed as for rhesus immunized while the few preeclamptic patients (8 pts) have values in the upper part of the range. Maternal serum creatinine was ≤ 0.9 mg in 4 of these patients and was not examined in the re-

maining 4 mothers with mild preeclampsia. This was not of a degree likely to have any effect on renal function. There is no correlation between α fc and the weight of the fetus (Fig 2).

The L/S ratios demonstrate the normal increase according to gestational age (Fig 3) and the α fc values reach the considered critical value of about 3% weeks. However there are great individual variations. With a gestational age of 34 weeks the L/S ratio was 1.9 in one sample and 2.1 in the remaining 27. On the other hand a L/S ratio of 0.1 was not found in the 49 specimens taken prior to 34 weeks. No special trend was found for L/S ratios among the different categories of patients.

There is a positive correlation between α fc and L/S ratio. The numeric variation in L/S ratio is much greater than in α fc and in Fig 4 the correlation is illustrated on a semilogarithmic scale. Kendall's correlation coefficient between α fc and L/S ratio was calculated to 0.56 which is highly significant.

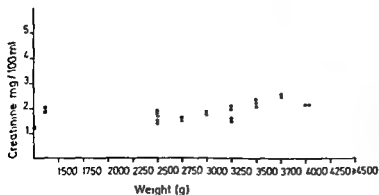


Fig 2 Creatinine concentration in samples of amniotic fluid obtained one week of delivery related to the weight of the newborn. ● One sample from a fetus with no growth retardation. ○ One sample from a growth retarded child (below 10th percentile—see text).

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Daktar^{DE} vaginalkram

Nytt antimykotikum för candida- vaginiter/vulviter

- Fungicid effekt
- Klinisk och mykologisk utläkning
i cirka 90% oavsett predisponerande faktorer
som diabetes, graviditet, samtidig p-piller- eller antibiotikabehandling
- Symtom som sveda och klåda försvinner eller lindras vanligen efter
2-3 dagar
- Olja i vattenemulsion – avtvättbar, färgar ej hud eller kläder

Pass-test

DAKTAR[®]

Vaginalkram 2

Bred spektrumantimykotikum för gynekologiskt bruk

Deklaration

100 g kräm innehåller

Mix-naz linstras g

Tef 46 63 Labrafilm 144 CS[®] paraffinum

liquidum acidum benzalkoniumbutylhydrsammansättning

et aqua purificata g

Figur 1 kaper DAKTAR vaginalkram innehåller som aktiv substans mix-naz i ett smältbart färdigt var. Mix-naz i har sålts in i ett smältbart färdigt fungicidiskt medel i ett färdigt paraffin svampar

DAKTAR färdigberas i mycket ringa grad i kal applikation

DAKTAR har goda kemiska egenskaper och innehåller ej färgämnen eller parabener. Ett färdigt avseendefärdigt har färdigberats. Kräm färgar ej kläder rött och kan avtvättas med tvål och vatten

Klinisk DAKTAR är effektiv i vaginaliter och vulviter orsakade av Candida och andra svampar

ter. Såväl mykologisk som klinisk utläkning har konstaterats i ca 90% av de behandlade fallen. Vid behandling med DAKTAR erhålles en snabbt insättande effekt med snabb lindring av symtom som sveda, klåda och flulor. Behandling med DAKTAR sänker förhöjda vaginala pH-värden vilket har en gynnsam effekt på den naturliga bakteriefloran till att DAKTAR lämpar sig väl för behandling av symtom som sveda och klåda hos gravida som prövas i en klinisk studie. DAKTAR lämpar sig även för behandling av barnet

Indikationer Vaginit och vulviter orsakade av svampar

Överdosering I sällsynta fall (mindre än 1%) har läst i kaliter till napparter

Dosering En färdig applikation (ca 5 g kräm 100 mg mix-naz) två gånger i slutet av dagen i 14 dagar. Det är viktigt för ett gott behandlingsresultat att patienten genomgår hela behandlingen

en Inget uppehåll behövs efter behandlingen

Situationer Vid graviditet bör applikation av DAKTAR inte göras

Applikation och bruk av DAKTAR medföljer varje förpackning

Förpackningar Tuhå 78 g med applikation



För närmare information kontakta

JANSSENS DIVISION AB Leo
Fack 251 00 HELSINGBORG

PRESENCE OF ANTI-c IN THE SERUM OF 42 WOMEN GIVING BIRTH TO c POSITIVE BABIES SEROLOGICAL AND CLINICAL FINDINGS¹

Jorunn Astrup and Lef Kornstad

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National Institute of Public Health Oslo Norway*

act During routine ante natal testing during the
1953-73 anti-c was found in the serum of 63 wom
twenty four of them had received blood transfusion
ously and of these 22 were multigravidae. In 39 wom
pregnancies alone were responsible for the immuni
2. The anti-c titres of the 42 women giving birth to c
ve babies showed that the probability of developing
olytic disease increases with higher titres. But low
by no means exclude the existence of such disease
ng the 43 c positive babies 32 had a positive direct
lobulin test. Some of the affected infants had a seri
degree of haemolytic disease.

reporting to the clinicians the finding of anti-c in
of pregnant women we sometimes feel that the
information is not taken as seriously as desirable
ever looking through the literature we found
ively few data on the clinical importance of this
body in pregnancy. Most of the available infor
ion comes from reports on a limited number of
s of haemolytic disease of the newborn (1, 2, 4
A greater number of cases was described by
ner et al (7). However such cases are some
s selected. They will therefore not give a true
ure of the clinical importance of anti-c in preg
cy how often the antibodies lead to haemolytic
ase of the newborn (HDN) the degrees of se
ity of the disease and the prognosis in the af
fected infants.

We therefore analysed our cases of anti-c found
routine antenatal testing during the period

1953-73. We here tried to find out whether the anti
body formation had been stimulated by previous
blood transfusion or pregnancies and looked for a
correlation between the maternal antibody titre and
the occurrence of HDN. In the affected babies we
attempted to estimate the degree of the disease on
the basis of the available serological and clinical
information.

MATERIALS AND METHODS

In our routine antenatal serological testing all blood sam
ples are tested not only for anti D but for other irregular
blood group antibodies as well. Thus all sera are ex
amined with saline albumin indirect antiglobulin and en
zyme methods against the Rh antigen c. We collected from
our laboratory records for the period 1953-73 the cases of
anti-c diagnosed during pregnancy.

When Rh antibodies of any specificity are demon
strated a blood sample is obtained from the husband for
determination of the probable Rh genotype. All cases
where the husband was c negative and the antibody could
not have any bearing upon the pregnancy were excluded
from the present study. So also were cases where addi
tional blood group immune antibodies were demonstrable
since it would be difficult to assess the relative importance
of the different antibodies.

Our series consists of 63 women representing 111 preg
nancies with anti-c. Two pregnancies ended in early
abortion. The deliveries took place at a number of hospi
tals. We obtained information on the babies in 61 of the 67
cases. The clinical data received were heterogeneous and
in some cases rather inadequate. Forty two c positive
babies in whom the results of the direct antiglobulin test
are known will be described in this paper.

Four infants known to be c negative and 11 with an
unknown Rh genotype were excluded. So also
positive baby in whom the result of the direct

¹ preliminary report of the study was presented at the
Congress of the International Society of Blood Trans
on Helsinki 1975.

Table I Previous blood transfusions in 63 pregnant women with anti-c

Antibody detected in pregnancy	Transfused women	Non transfused women	Total
No 1	2	0	2
No 2	5	8	14
No 3	4	9	13
No ≥4	12	22	34
Total	24	39	63

test was not available this baby gave no evidence of haemolytic disease. Among the 14 infants with unknown Rh genotype eight had a negative direct antiglobulin test. In the remaining six the outcome of the test is unknown. However, one of these babies reportedly had HDN.

When antibody titres are given in the sequel it unless otherwise stated always refers to the results of the indirect antiglobulin test performed on *cde/cde* cells.

RESULTS

Among the 63 pregnant women with anti-c 24 had a history of previous transfusions (Table I). Only 2 of the 63 cases were diagnosed during the first pregnancy. Both women had been transfused. In 47 women antibodies were first observed in the third or a later pregnancy. Only one third of these women had been transfused while the remainder were immunized as a result of pregnancy and delivery.

Of the 42 babies known to be c positive 32 had a positive direct antiglobulin test and HDN. In the last ante natal examination one day to 4 weeks before delivery the women with c positive babies had antibody titres up to 1024 in the indirect antiglobulin test (Table II).

The ten mothers with healthy infants all had titres of 8 or below. In two of them the antibody was demonstrable with enzyme treated cells only while the antibody in one case reacted with albumin and papain methods but not by the indirect antiglobulin technique.

Among the mothers of babies with haemolytic disease two had particularly high titres 512 and 1024. In the 30 others the titres ranged from 1 to 128 with a titre of 8 or lower in 14 mothers (Table II).

We received information about the haemoglobin

values on the first day of life in 21 of the 32 with haemolytic disease (Table III). In the 11 infants are grouped according to whether or not transfusions were given or not. The haemoglobin concentrations refer to cord blood except in 3 cases where the kind of blood examined is stated. Only one baby had a grave anaemia, six had intermediate haemoglobin values.

The 9 transfused babies were all born c+. The mother of the gravely anaemic infant had an anti-c titre of 512. This baby to whom we return in the discussion had in the cord blood haemoglobin concentration of 7.6 g and a haematocrit concentration of 11.4 mg per 100 ml. It received exchange transfusions but developed cerebral disorders probably caused by bilirubin damage. Two transfused infants with intermediate haemoglobin values recovered uneventfully. Maternal antibody titres were 4 and 64 respectively.

The 4 of the 9 transfused babies with a normal cord blood haemoglobin all developed pathological bilirubin values. Three of them recovered fully. The fourth receiving two exchange transfusions developed a pathological electroencephalogram. This was presumably caused by a temporary respiratory and cardiac arrest during the first transfusion. The antibody titre in the mother was 1.

Of the two transfused babies on whom we have no information about the haemoglobin values one died after exchange transfusion with an oxygenated blood of the Rh type *cde/cde*. The other recovered without complications.

Among the 23 non transfused infants one initially had a normal haemoglobin concentration but died. Autopsy revealed kernicterus, a pale liver, menovaginal and atelectasis of the lungs. The antibody titre of the mother was 8. The remaining babies recovered without sequelae.

Table II Occurrence of haemolytic disease related to anti-c titres in the mothers

	Titres by the indirect antiglobulin test			
	≤8	16-128	512-1024	≥1024
HDN	14	16	7	10
No HDN	10	0	0	0

III Exchange transfusions correlated to globulin concentration (g per 100 ml) on the day of life

	<10	13-15	≥17	Un known	Total
used					
s	1	2	4	2	9
transfused					
s	0	4	10	9	23
	1	6	14	11	32

DISCUSSION

present series of pregnant women with anti-c sensitization was in more than 60% of the cases caused by pregnancies and deliveries alone. As then to be expected the proportion of multiparous women is quite high. In two cases only the anti-c formation was almost certainly the result of previous transfusions alone. The antibody was not in the first blood sample drawn in the 3rd month of pregnancy respectively. In about 10% of the cases the immunizing stimulus may have been a previous blood transfusion or previous pregnancy or perhaps most likely both. Our findings are in agreement with those of Spielmann & Seidl in their cases of HDN caused by anti-c: the sensitization could not in any case be claimed to be the result of previous transfusion alone. The anti-c formation was either a result of previous pregnancies or of a combination of previous pregnancies and transfusions. As already pointed out (Table II) the anti-c titres were low in the mothers giving birth to infants not sensitized by HDN. Concerning the 32 babies with HDN the antibody titres were in 18 mothers 16 or higher, in 14 mothers 8 or lower. The great proportion of low-titred antibodies in this group is notable. In some cases the titres might have been higher if titrated at term. However, in 6 of these 14 cases a last titration was performed within one week before delivery and in 2 cases only the interval was as long as 4 weeks. It may thus be concluded that the probability that the babies will be affected increases with higher titres, low titres by no means exclude the existence of haemolytic disease. As previously mentioned we tried to exclude in the present series women who in addition to anti-c also had other blood group immune antibodies. In this connection the possible presence

of anti-E of course represents a special problem, as CCDEe (R₁R₂) cells were not always available. However, 22 of the babies with a positive direct antiglobulin test are known to be E-negative. Six infants are CcDEe (R₁R₂) and 3 have an Ee father but have not been E₁ grouped themselves. Among these 9 babies only one needed exchange transfusion, a possible simultaneous presence of anti-E cannot have contributed much to the severity of the disease in these infants.

In a single case anti-E is known to be present in addition to anti-c. This is the case of the gravely anaemic infant already mentioned. Four weeks before delivery the anti-c titre (against cde/cde cells) was 0 in saline, 128 in albumin, 512 by the indirect antiglobulin test and 256 against papainized cells. In the same sample the anti-E titre (against CDE/CDE cells) was 64 in saline, 64 in albumin, 32 by the indirect antiglobulin technique and 128 against papain-treated cells. The baby was at the hospital found to be C+ c+ D+ E-. However, at our laboratory the father's cells gave clear-cut negative reactions with several anti-e sera, suggesting an erroneous E typing of the infant. Anyhow, the anti-E of the mother seems to be predominantly IgM and the incomplete anti-c must be assumed to have the major responsibility for the grave haemolytic disease.

In the large series presented by Giblett (3) anti-c caused over 3% of the cases of HDN. With the marked decline in the total number of cases of HDN resulting from the current Rh(D) prophylaxis the proportion of HDN cases due to anti-c will increase. Although anti-c erythroblastosis most often is mild to moderate, severe cases with intrauterine anaemia may occur. Therefore, amniocentesis may be indicated to select cases where labour should be induced or intrauterine transfusion given.

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NEONATAL AND PROSPECTIVE FOLLOW UP STUDY OF INFANTS DELIVERED BY VACUUM EXTRACTION (VE)

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Forty infants delivered by vacuum extraction have been studied in the neonatal period—neurological examination, neonatal CSF-examinations, skull X ray examination, transillumination and sonoencephalography at 14 months of age—developmental and behavioural evaluation, neurological examination, skull examination, sonoencephalography and electroencephalography. Two infants died in the neonatal period, both cases a life threatening situation for the fetus. In immediate delivery, CSF cytological signs oforrhage were observed in 42% of the 26 infants who had a successful lumbar tap, compared to 10% found in al deliveries. The result of the neonatal neurological did not differ from that in a control group. The result of skull X ray examination and sonoencephalography also within normal limits. In the follow up study, visual problems were found in 25% but otherwise few abnormalities were found. The deviations found out for the present indicate any later signs of brain os. It is concluded that this prospective study has n that VE-delivery in fullterm babies seem to imply isk of serious cerebral sequelae. Further follow up es at a later age in order to evaluate the incidence of lled minimal brain damage in VE-delivered children quired.

the introduction of the vacuum extraction delivery method in Sweden by Malmström in 1949, the number of infants delivered by VE have numbered forceps deliveries (Table I). Earlier orted studies with regard to the outcome of -delivered infants have mostly been retrospec- e taking into account perinatal events sev- l years after the delivery (for review see (3)). pective studies with thorough perinatal obser- ions and examinations followed by repeated ex- minations during the first year of life are few. Consequently we have performed the present in- tigation in order to evaluate perinatal (obstetri-

cal and neonatal) events at VE-deliveries prospec- tively in relation to later development and long term sequelae.

MATERIAL

The material comprises a consecutive series of 48 instru- mental deliveries (VE and/or forceps) out of 1169 live births during a 4-month period 1971-1972. In 4 cases forceps were applied. In 4 deliveries forceps were used because the vacuum extractor became detached. The present report deals with the 40 infants delivered by VE only.

The mothers were between 19 and 40 years of age (mean age 27 years). Thirty-one mothers were primi-gravidae. The gestational age at birth was between 38 and 41 weeks in 27 infants, more than 41 weeks in 9 infants and 37 weeks or less in 4 infants. There were 22 girls and 18 boys. Five infants were large for gestational age and one infant small for gestational age.

The material was divided into 2 groups according to the indication for VE-delivery (Table II). *Fetal indication* i.e. irregular fetal heart rate ($n=18$). *Maternal indication* i.e. standstill in labour or some other obstetrical factors as shown in Table II ($n=22$).

The number of low and mid VE did not differ between the two groups. High VE was never used during this study. The control group for the neonatal neurological study included 28 full term neonates with a normal pre- and perinatal period and non-instrumental delivery.

Parental and institutional consent had been obtained for the examinations performed in the postnatal period in every infant included in this study. χ^2 test was used for group comparisons.

Part I Neonatal study

METHODS

Neurological examination. In the schedule for the neonatal neurological examination 21 items were used.

Table 1 *Incidence of forceps and vacuum extraction (VE) deliveries in the Obstetrical Department of the University Hospital Lund*

	Period		
	1950-1954	1960-1964	1975
Forceps	2.5%	0.7%	0.6%
VE	-	2.6%	3.9%
Total number of deliveries	11 801	13 766	3 578

Palmar grasp
 Plantar grasp
 Babinski's sign
 Withdrawal reflex
 Rooting response
 Sucking response
 Facial movements
 Traction test
 Head control in sitting posture
 Head movement in prone position
 Crawling in prone position
 Stepping movements
 Moro response
 threshold
 abduction-extension
 abduction-flexion
 Rotation test
 Doll's eye test
 Muscle tone
 or activity
) type
 amount
 Sensitivity to touch and stimulation

The examination was performed on the third day of life 2 hours after a meal and in adequate state according to Prechtl & Beintema (11). Optimal result for each item was scored one. The scores were added up to a total score and thus the maximum obtainable value will be 21. According to the total score the material was divided into quartiles.

At the neurological examination the skull was examined for external traumatic scalp deformity and excoriation of the skin. Measurements of head circumference and transillumination of the skull were also made.

Cerebrospinal fluid (CSF) examination. Cytological CSF examination was performed on the third

day of life according to the method of Sorsby. This method involves a one hour CSF sample in a glass cylinder fixed by on a microscopic slide and thereafter the supernatant fluid is gently sucked away the cylinder moved the preparation air-dried and stained finally examined in a light microscope. Presence of erythrophages and siderophages is considered indicative of cerebral or spinal haemorrhage.

The content of lactate and pyruvate in CSF measured enzymatically (15). The lactate/pyruvate ratio is indicative of the cerebral energy status thus a tool for measuring cerebral oxygen depletion.

Sonoencephalography was performed on the third day of life. The mid-echo position was determined and the lateral ventricular index calculated according to Sjögren (12). *X-ray examination* of the skull was performed on the first or second day after birth. The methodology and results are presented in a follow up study (part II).

RESULTS

Two VE delivered infants died in the neonatal period. One infant with birthweight 1350 gram, first of twins, was delivered in 31st week of gestation by VF because of impending twin interlocking. The baby cried immediately after birth but became apnoeic and required assisted ventilation. The infant died at 18 hours of age and at autopsy bleeding in the galea, cerebellar haemorrhage, a tentorial tear and cerebral cellular anoxic damage were found. The second infant was a term rapidly extracted with a vacuum extractor because no fetal heart activity could be recorded when the mother arrived in the labour ward. The baby was resuscitated but died on the second day of life after periods of fits and apnoea. Autopsy showed

Table II *Indications for VE deliveries*

Fetal indication	18
Irregular fetal heart rate	17
Impending twin interlocking	1
Maternal indication	21
Secondary uterine inertia	14
Prolonged second stage	3
Toxaemia	2
Epidural anaesthesia	1
Previous vaginal repair	1
Retinal ablation	1
Total number	40

vein bleedings and cerebral ventricularorrhage. These two cases are not included in the following account of the neonatal study results. Among the 38 surviving infants 5 had Apgar scores at one minute after birth below 7 i.e. early neonatal asphyxia was encountered in 13% of the 38 VE delivered infants. On 4 of these 5 infants mid VE was applied—in two infants for a medical indication—and on the fifth baby low VE for a medical indication.

The total scores of the neonatal neurological examination are presented in Fig. 1. No statistically significant differences were found between the VE delivered infants and those in the control group. Dividing the VE infants into two groups (lower quartile and upper three quartiles) according to total scores, no significant correlation could be found either with the indication for VE (maternal or fetal) or the type of VE (mid or low).

The CSF cytological examination showed signs of hemorrhage (cerebral or spinal) in 11 of 26 infants. In 12 babies this examination could not be performed because of parents' refusal or was incomplete because of unsuccessful lumbar puncture or laboratory errors. In the abnormal CSF samples, often only a few erythrocytes and siderocytes were seen, i.e. the number of pathological cells was usually not abundant. No significant correlation between the CSF cytological findings and the indication for or type of VE or the neonatal neurological examination was found.

The CSF lactate and pyruvate analysis was performed in 31 babies. The lactate/pyruvate ratio was slightly increased in 2 babies—20.9 and 18.9 respectively—as compared to the upper normal value of 17.6 for this age group (15).

Sonoencephalography performed in 35 infants showed a ventricular index within normal limits, i.e. ± 0.03 according to Sjogren (12) and no abnormal midline echo deviations. No skull fractures were found.

Part II Follow up study

METHODS

At 4 months of age re-examination was carried out in the surviving infants. Three infants had left the area leaving a total number of 35 children in the follow up study. A parental interview with special attention directed to problems concerning sleeping and feeding habits, colic, irritability, sensitivity to sudden sounds, developmental

Neonatal neurological exam. 14 days of age

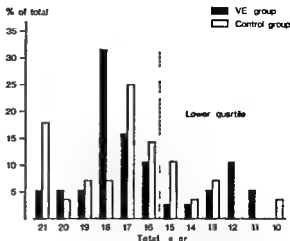


Fig. 1 Total scores of the neonatal neurological examination.

milestones and motor aberrations was performed for every VE-delivered infant.

A conventional neurological examination including evaluation of gross and fine movements, speech development as well as head circumference measurement and skull transillumination were performed. In addition EEG was performed in 34 infants with sleeping records obtained in two-thirds of the infants. Sonoencephalography was performed in 33 infants as described in the neonatal study (part I).

X-ray examination of the skull. On the radiograph measurements were made of the greatest length (L), width (W) and height (H) between the internal tables of the skull. H is the maximum perpendicular distance between the vault of the skull and the line from the nasion to the posterior margin of the foramen magnum. The maximum distance between the inner margins of the mandibular necks was also measured (M). The cranial index (C index) was determined as $(H+L+W)/M$. However, due to the normally existing deformity of the skull in the first days after birth, only W/M index could be calculated on the neonatal radiographs of the skull. The W/M indices from the neonatal and the follow up study could be directly compared, whereas the sum of H+L+W and the C index from the follow-up study were compared with known normal values (4).

RESULTS

In the 35 infants studied at 14 months of age the neurological examination showed no abnormalities. EEG was slightly abnormal in 2 infants showing unilateral sporadic focal sharp-waves during sleep in one infant and asymmetry of the slower components in the other infant. Sonoencephalography and

Table III *Abnormalities in the 14 months of age follow up study (part II) in relation to VE-indication, cytological examination and neurological examination in the neonatal study (part I)*

N=number of infants examined at 14 months of age and number of infants VE-delivered on fetal indication
 abnormal CSF cytology or neonatal neurological examination respectively out of total number of infants
 exam =CSF sample not obtained

Examination at 14 months of age	N	35/38	Fetal VE indication (irregular fetal heart rate) 16/38	Neurological score at 3 days of age within lower quartile 10/38	CSF , of age 11/16
Abnormal EEG	2	2		2	1 (not exam. 1)
Increased sound sensitivity	3	2		3	2 (not exam. 1)
Behaviour problems	9	2		3	1 (not exam. 3)

X ray examination of the skull showed no abnormalities in any infant

In the parental interview the mothers of 9 infants stated single or multiple complaints including disturbed sleeping habits (5 cases) colics (6 cases) breath holding spells (2 cases) and unusual sound sensitivity (3 cases). The last mentioned complaint was confirmed at the examination. One of these three infants also had breath holding spells and another had asymmetrical EEG as mentioned above.

Correlation to neonatal study (part I) The main abnormalities of the follow up study as related to neonatal observations and examinations are presented in Table III. The infants with abnormal EEGs were both delivered by VE for a fetal indication—one with low and the other with mid VE—and belonged to the lower quartile in the neonatal neurological examination. One of these babies also had CSF cytological signs of haemorrhage while lumbar puncture was unsuccessful in the other baby.

Only 2 of 9 infants with parental complaints of behaviour problems were delivered by VE for a fetal indication and only 3 of these 9 infants belonged to the low quartile in the neonatal neurological examination study. Furthermore CSF examination performed in 11 of these 9 infants showed haemorrhage in only one baby.

The results of X ray examination of the skull are shown in Table IV. Three groups are considered here: infants with CSF signs of haemorrhage, infants with normal CSF and infants in whom no CSF examination had been performed. The calculated indices did not show any difference between the three groups or from normal values. Normal X ray

examination indices were also found in infants belonging to the lower quartile of the neonatal neurological examination.

DISCUSSION

The necessity of taking into consideration the obstetrical situation when evaluating the consequences of VE-delivery must be stressed. In the present series two infants died after VE-delivery. In both VE was applied because of a threatening intrauterine situation. One infant delivered by VE because of impending respiratory distress locking several weeks before term. It has been stated that VE application in preterm delivery should be avoided (10) and in this case, perhaps, it might have been preferable. Thus in the two cases the risk of severe complication was considerable already before the VE application and the method per se might not be the only reason for the unfortunate outcome in these cases.

Earlier investigations of cerebral haemorrhage after instrumental deliveries are based on different findings. In these studies the indications for instrumental aid have mostly been fetal asphyxia and the results are inconclusive (8). In the present series CSF cytological signs of haemorrhage were found in 42% (11 of 26 cases) of the surviving infants and in equal frequency after fetal and instrumental indications. These results should be compared with the 10% found in normal deliveries (16) and the 40% found in infants with respiratory distress syndrome (1).

Predictions from the results of the CSF examination must for the present be done cautiously. Abnormal cytological findings have been shown to be

IV Skull X ray examination (mean) in relation to CSF cytology

al values according to (4)

	Neonatal study (age 1-2 d) W/M index	Follow-up study at 14 months of age		
		W/M index	Sum of H+L+W	C index
series	17 2 n=33	17 9 n=28	453 n=29	57 4 n=29
signs of hemorrhage	17 5 (16 0-19 0) n=11	17 9 (16 8-18 4) n=8	450 (430-476) n=8	57 9 (54 0-60 1) n=8
al CSF cytology	17 2 (16 4-17 9) n=15	18 1 (16 9-19 2) n=11	457 (435-490) n=12	58 1 (53 9-60 4) n=12
SF examination	16 8 (16 1-17 6) n=12	17 5 (16 1-18 7) n=9	444 (419-469) n=9	56 2 (53 1-59 5) n=9
al values	17 3±0 6		441±45	56 0±6 0

mon and of uncertain prognostic value in un-
 plicated concussion in adult patients (14)
ever after perinatal asphyxia we have found
 cytological CSF examination has a prognostic
 e i.e. normal cytology seems to imply a better
 prognosis as compared with abnormal cytology

In the present investigation there were no cor-
 relations between the CSF cytological finding and
 other neonatal parameters. The results from the
 months examination are also inconclusive with
 respect to the interpretation of the cytological re-
 sults. Long term follow up studies over several
 years are required to clarify whether any minor
 cerebral dysfunction could be related to the abnor-
 mal CSF cytological findings in the 42% of VE
 delivered infants.

The possibility of spinal bleedings as a source for
 pathological cells in the CSF should also be kept
 in mind when discussing neonatal traumatic nerv-
 ous system lesions. Towbin (17) has pointed out
 that such lesions could easily be overlooked. How-
 ever there were no neurological signs indicating a
 spinal injury.

The small deviations in the two abnormal EEG
 recordings as well as the two slightly elevated CSF
 lactate/pyruvate ratios do not permit any conclu-
 sions. Earlier EEG studies have shown disparate
 results mainly because of differences in selection of
 neonatal VE indications and age at examination (2,
 8). CSF lactate/pyruvate studies after VE
 delivery have not been presented before in the liter-
 ature whereas blood lactate and pyruvate analyses
 have been found to be normal after VE-delivery (5).
 The normal results of the X ray examination of

the skull are in agreement with Hickl (8) while
 Backman et al. (2) reported 9 instances of skull
 lesions in a series of 101 VE-deliveries. However 7
 of these cases were high VE.

Important questions arise concerning the infants
 with behaviour problems. Is the behaviour of these
 infants really deviating from normal (and if so be-
 cause of neonatal lesions?) or are their mothers
 more anxious and reacting with great alarm to nor-
 mal signals of discomfort in their infants? It should
 be noted that 7 of 9 infants with behaviour problems
 had been delivered because of maternal indications
 (most often secondary uterine inertia). The present
 study does not allow any definite conclusion al-
 though a possible causative mechanism should be
 kept in mind. Anxiety during delivery may threaten
 the fetus through maternal hyperventilation causing
 secondary blood gas changes in mother and
 placenta resulting in fetal hypoxia (7). More exten-
 sive studies are needed to clarify the complicated
 relationships between mother and infant both be-
 fore and after birth.

Taking into account the total strain sustained by
 the fetus during delivery and the findings at 14
 months of age it seems to us that VE-delivery is a
 rather safe and innocuous method of delivery when
 applied at full term and according to present obstet-
 rical practice. This conclusion is in agreement with
 earlier reported follow up investigations (2, 3, 6,
 18).

The abnormalities observed in the neonatal study
 and in the follow-up study at 14 months of age
 warrant further follow-up studies of these children
 at school age in order to evaluate whether

dence of so-called minimal brain damage (MBD) symptoms is higher in VE-delivered children than in children after non instrumental deliveries

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EFFECTS UPON THE FETUS OF OXYGEN ADMINISTRATION TO THE MOTHER

A Study in Monkey

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Catheters were placed into assorted arteries and
of 8 anesthetized pregnant monkeys and their
es Oxygen sensitive electrodes were also inserted
simultaneously into 3 of the 8 fetuses Periodic samples of
maternal and fetal blood were analyzed for PO_2 , PCO_2 and
Oxygen administration to the mothers reliably in-
creased the PO_2 of blood taken from the fetal carotid
artery and less constantly augmented the PO_2 of blood
drawn from the femoral artery and vein During 5-6
days of study the oxygen tension of fetal blood samples
from all animals progressively declined However the most
marked declines in PO_2 values at all fetal sites were regu-
larly observed at those times as—or after—the mothers
were removed from anaesthesia At these times also the
magnitudes of the increases in fetal blood PO_2 brought
about by administering oxygen to the mothers diminished
markedly and in parallel at all sample sites The closely
similar magnitudes of these various reductions in all fetal
sample sites indicate that the basic mechanisms leading to
reduced oxygen delivery lie outside the fetuses and are
most likely due to decreased maternal blood flow to the
fetuses because of increased maternal sympathetic stimula-
tion These reductions in oxygen delivery to the fetus are
regularly reversed by reanaesthetizing the mothers
Studies carried out with oxygen sensitive electrodes
demonstrate that administering oxygen to the mothers
markedly increases oxygen tension of fetal tissues but after
1 sec delay

19) On the other hand numerous studies have
shown that administering oxygen to the mother
actually improves rather than diminishes the ox-
ygenation of the fetus (4 12 17 20 21 22 25 26)

The present study uses a rhesus monkey model in
order to look further into this important question
Catheters were placed in blood vessels of mothers
and fetuses Blood samples were withdrawn from
both and their oxygen tensions compared at dif-
ferent times and under different physiologic condi-
tions Particular attention was paid to defining any
differences in oxygen transfer to the fetuses accord-
ing to depth of anaesthesia of the mothers Oxygen
sensitive electrodes inserted into fetal subcutane-
ous tissues permitted tissue oxygen tension to be
followed on a moment by moment basis The time
relations between administering oxygen to the
mothers and observing changes in oxygen tension
of fetal tissues were determined

MATERIAL AND METHODS

Eight pregnant rhesus monkeys near term (154-158 days
of gestation) were used Pregnancy length was known with
an accuracy of ± 2 days Body weight varied between
6.2 and 9.4 kg All animals were anaesthetized with
pentobarbital 35 mg/kg I V A warm water mattress was
placed beneath the mothers and an overhead heat shield
servo-modulated by a rectal thermistor maintained the
mothers body temperature within the range 37°-38°C
The mothers left femoral artery and vein were exposed
and polyethylene catheters were inserted advanced to
the level of the midabdomen and fixed in place A
hysterotomy was performed and the left leg of the fetus

the question whether administering oxygen to
mothers in labour improves oxygenation of the fetus
has remained unanswered It has been suggested
that 100% oxygen breathing by the mother may
actually reduce rather than increase the oxygen
supply to the fetus because oxygen at high concen-
trations may constrict uterine blood vessels (10 11)

Table 1 pH Determinations during periods of oxygen administration and air breathing in full term monkeys

MFA=maternal femoral artery MFV=maternal femoral vein FCA=fetal carotid artery FFA=fetal femoral artery FFV=fetal femoral vein

	100% O ₂ Air		100% O ₂ Air		100% O ₂ Air		100% O ₂ Air		100% O ₂
MFA	7.40	7.41	7.45	7.42	7.45	7.435	7.465	7.49	7.41
MFV	7.35	7.38	7.39	7.40	7.40	7.41	7.43	7.46	7.40
FCA	7.32	7.34	7.36	7.33	7.34	7.355	7.33	7.37	7.29
FFA	7.31	7.33	7.35	7.32	7.32	7.35	7.325	7.35	7.30
FFV	7.285	7.31	7.32	7.32	7.32	7.31	7.29	7.325	7.24

was identified and externalized the inguinal region incised and the femoral artery and vein isolated. Polyethylene catheters were inserted into both vessels their tips advanced to the level of the upper abdomen and they were secured in place. The fetal inguinal incision was repaired the leg restored in the uterus and the fetus still within the uterus was rotated through 180°. The fetal head was externalized the right carotid artery isolated through a ventral midline neck incision and a polyethylene catheter was inserted and fixed in the right carotid artery so that its tip lay in the ascending aorta. The neck incision was repaired and the head replaced into the uterus. A polyethylene catheter was inserted into the uterine cavity and the maternal abdominal walls were closed. Lost amniotic fluid was replaced by 40 ml of warm saline injected through the intrauterine catheter.

The mothers were next placed on their side. The catheters passing from the blood vessels of the mothers and fetuses were attached to Statham pressure-sensitive transducers and the blood pressures and heart rates of the mothers and fetuses and the intrauterine pressures were recorded on a Brush multichannel polygraph. Small blood samples (0.4 ml) were periodically withdrawn from all catheters and analyzed for pH, PO₂ and PCO₂ using the trip apparatus (Radiometer Copenhagen). Accuracy analysis was reliable with a maximum variance of 1.5%.

The fetal catheters were so located as to provide samples of blood of the composition which normally supplies the head (withdrawn from the carotid artery catheter) and the body (withdrawn from the femoral artery catheter). Fetal blood volume was maintained by replacing all blood withdrawn by equivalent volumes of maternal blood. The procedures used in animal preparation have been described elsewhere (1).

The mothers breathed spontaneously throughout the period under investigation. A 3 litre clear plastic bell was placed over their heads and alternately flushed with 100% oxygen and with room air—30 min each. At the end of each period of oxygen or air breathing blood was withdrawn from all maternal and fetal sample sites. Thus two sets of PO₂ and pH values of mothers and fetuses from all sample sites were compared each hour—one set was obtained while the mothers were breathing 100% oxygen and the other while they were breathing air. The repeated blood sampling during alternate oxygen and air breathing for up to 6 hours permitted fetal oxygenation to be followed at times when the mothers were successively under

1) deep anaesthesia and 2) early and 3) late anaesthesia emerged from anaesthesia 4) while nearly awake strained on the operating table and 5) after of anaesthesia with pentobarbital injection, 30 mg. Only those periods were evaluated during which the fetal arterial blood pH values remained above 7.30. Thus stringent restriction ensured that all values reflect findings obtained from metabolically stable fetuses.

In three of the pregnancies oxygen-sensitive electrodes were utilized to continuously record the oxygen tension in fetal tissue. The 3 mothers and fetuses were prepared as described above and in addition miniaturized Clark needle electrodes (outside diameter=0.5 mm (2)) were inserted 1–2 cm into the subcutaneous tissue of the fetus just before head reinsertion. The leads from electrodes were connected to a Radiometer PO₂ measuring module (Digital Acid-Base Analyzer-PHM 61) and output was monitored on a Brush polygraph channel with blood pressures and heart rates of mother and fetuses and intrauterine pressure. The electrodes calibrated just prior to tissue insertion by adjusting meter settings with the electrodes alternately placed in Radiometer PO₂-zero solution and in water saturated with air. All tissue PO₂ measurements were carried out within 45 min of the last electrode calibration. During the electrode studies the mothers breathed either 100% oxygen or room air for 2–6 min at a time. Alternations in oxygen tensions in the fetus were continuously followed as they were recorded on the polygraph. The abruptness and termination of oxygen breathing by the mother permitted the time lag in the elevation or reduction of the fetal tissue PO₂ to be determined without the limitation imposed by the time constant of the measuring system.

RESULTS

The effects of depth of maternal anaesthesia and magnitude of oxygen transfer to the fetus were specifically studied in the present investigation. The mothers were first anaesthetized with pentobarbital 35 mg/kg. Then they were permitted to awaken over the next 3 hours. After study for four hours while nearly awake they were again fully anaesthetized and followed during a final hour.

PO₂ Determinations in maternal and fetal blood during oxygen and air breathing

maternal femoral artery MFV=maternal femoral vein FCA=fetal carotid artery FFA=fetal femoral artery fetal femoral vein

Deep anaesthesia				Semi awake				Deep anaesthesia	
100% O ₂		Air		100% O ₂		Air		100% O ₂	
450	93	420	89	470	80	370	83	290	91
53	51	57	52	65	45	47	41	89	53
34	28.5	33	22	27	25	20.5	19	25.5	16
28	23	23	18	23	21	17.5	16	21.5	16.5
21	18	17	12	14	13	8.5	8.5	-	12

oxygen tension of fetal blood samples varied a wide range during the 5-8 hours of study and be related to the depth of anaesthesia of the mothers. However, it also varied in relation to the on of the study itself and to a number of other factors. Because of the multiplicity of factors affected oxygenation of the fetuses, comparison of oxygen values obtained from different animals at different times were not practicable. None the less, the general findings obtained with the various animals in the present study accord with the results observed on many other animals followed in previous studies both with respect to trends of the changes observed and with respect to the absolute values of these changes. Because of this overall consistency of results, the findings obtained with a single mother-fetus pair (mother 790H) are presented as representative of the findings obtained with all animals.

The value of a fetal blood sample serves as a valuable indicator of the respiratory and metabolic state of the fetus at that time. As long as the maternal and fetal blood pH values remain within their normal ranges, it may be inferred that the fetuses are neither retaining carbon dioxide nor releasing excessive amounts of lactic acid. Table I presents the pH values of blood samples taken from the various sample sites of mother 790H and her fetus at every stage throughout the study. As can be seen, these values generally remained within their normal ranges. Thus, whatever the changes brought about in oxygen tension of the fetus in this study, they were never sufficient to lead to metabolic disturbances.

The initial and terminal arterial blood pH values of mother 790H were 7.40 and 7.47. Thus, this mother maintained her arterial blood pH values relatively constant throughout the study except when

she was nearly awake when the pH augmented to 7.49 due to hyperventilation. The initial and final maternal femoral vein blood pH values were 7.35 and 7.44. These values were generally about 0.03 units lower than were the values of samples drawn from the femoral artery. The initial and final pH values of blood taken from the fetal carotid artery were 7.32 and 7.31. Again, throughout the study the fetal carotid artery blood pH remained about 0.01 units higher than the pH of blood sampled from the fetal femoral artery. Similarly, the fetal femoral artery blood pH remained about 0.02-0.03 units higher than the pH observed in fetal femoral vein blood.

The oxygen tensions of blood samples taken from the various blood vessels of mother 790H and her fetus are detailed in Table II and the values obtained from the various sample sites of the fetus are illustrated in the bar graphs of Fig. 1. The PO₂ values of the maternal arterial blood when the mother was breathing room air generally fell within the range 90-95 mmHg, and when she was breathing 100% oxygen within the range 410-490 mmHg. Thus, mother 790H adequately ventilated her lungs whatever her depth of anaesthesia. Paradoxically, as mother 790H emerged from anaesthesia, the PO₂ values of her arterial blood lowered both when she breathed oxygen (to 290-370 mmHg from 420-450 mmHg) and when she breathed room air (to 80-83 mmHg from 91-93 mmHg). Furthermore, these depressions in arterial blood PO₂ occurred despite heightened respiratory efforts. When 790H was nearly awake, the oxygen tensions measured in venous blood also decreased by 6-10 mmHg as did the PO₂ values of blood samples taken from all sites of the fetus.

The blood sampled from the maternal inferior vena cava (through the femoral vein catheter)

OXYGEN TENSIONS OF BLOOD SAMPLES TAKEN FROM CAROTID AND FEMORAL ARTERIES AND FEMORAL VEIN OF FETUS WITH MOTHER ALTERNATELY BREATHING OXYGEN AND ROOM AIR (30 Minutes Each)

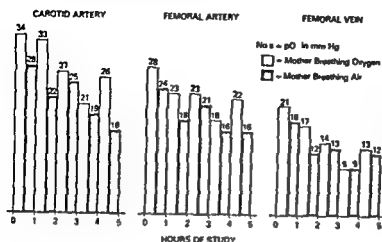


Fig 1 Bar graphs showing oxygen tensions of successive blood samples taken from carotid and femoral arteries and the femoral vein of the term fetus of monkey 790H times when she was alternately breathing 100% oxygen and room air for 30 minutes over a 5 hour period

showed considerably higher PO₂ values than did blood sampled from any fetal site at all sample times. The average difference in PO₂ value of maternal vena cava blood and of carotid artery blood of fetus was 28 (range=19-43) mmHg. The magnitude of this difference varied during the experiment and tended to be largest when the mothers were deeply anaesthetized and smallest when they were awake. In addition the PO₂ values of all fetal blood samples generally declines during the study. Thus as can be seen from Table I and Fig 1 the PO₂ value of the carotid artery blood of the fetus of 790H

declined from 34 to a final 26 mmHg when sampled breathing 100% oxygen breathing and from 28 to 16 mmHg when sampled during room air breathing.

Similar decreases in PO₂ were observed in blood samples taken from all other fetal sites, i.e. the fetal femoral artery blood PO₂ declined from 28 to 22 mmHg during 100% oxygen breathing and from 24 to 16 mmHg when the mother breathed room air and the fetal femoral vein blood PO₂ declined from 21 to 13 mmHg during 100% oxygen breathing and from 18 to 12 mmHg when the mother breathed room air. A large decrease in PO₂ of fetal blood samples took place during the second hour of the experiment. This decline was observed first in the posterior circulation of the fetus, i.e. in the femoral artery and vein blood samples and later in the circulation to the head (in carotid artery blood samples).

The lowest PO₂ values of fetal blood samples generally occurred when the mothers were semi-

awake on the operating table. At this time the PO₂ values of blood samples taken from the carotid artery, femoral artery and femoral vein of the fetus of the mother 790H with the mother breathing oxygen were 21, 18 and 9 mmHg and with her breathing room air 19, 16 and 9 mmHg respectively. After the mothers were reanaesthetized the PO₂ values of all fetal blood samples elevated. In fetus of monkey 790H the early PO₂ elevations observed after the mother was reanaesthetized were 4-5 mmHg. In other fetuses these elevations were usually larger (as great as 8-10 mmHg). The improvement in the oxygenation of the fetus of 790H largely disappeared by the time of the second sampling (after 1 hour).

The fetal carotid artery blood samples recorded showed higher PO₂ values than did samples taken from the femoral artery. The average difference through the experiment was 4.2 mmHg. However this difference was larger early during the study (when it was 6-10 mmHg) than at the end (when it was 0-4 mmHg). The final samples examined showed no differences in PO₂ value between carotid and femoral artery blood. At this time oxygen transfer from the mother to fetus was greatly reduced and the PO₂ values of all fetal blood samples were greatly depressed.

The differences in PO₂ value of fetal blood samples when the mother was breathing oxygen compared to those when she was breathing room air at different times during the study are illustrated in Fig 2 as they were observed in mother 790H.

tus At the time of the first comparison sub-
on of oxygen for air breathing caused the PO_2
d carotid artery femoral artery and femoral
lood to augment by 6.4 and 2 mmHg respec

When the same values are compared during
ard and fourth hours as the mother emerged
anaesthesia and early during the fifth hour
she was largely awake the corresponding
s were 2.2 and 1.4 -4 and -4 and 2.2
mmHg respectively Subsequently after the
er and her fetus were reanaesthetized sub-

of oxygen for air breathing caused these
s to again augment to 7.1 and 4 and 10.1 and
nHg respectively In interpreting the differ

in arterial blood oxygen values of the fetus at
ent times it is important to bear in mind that
intervals over which the mothers breathed ox

and room air from one sample time to another
long (30 min) This time length is of sufficient

tion that substantial physiologic changes may
r both to mother and fetus and these changes
alter the base line serving for comparison from

interval to the other In fact substantial
iges in physiologic state of the mothers and
ies are expected in the present study since such

iges were purposely induced by first injecting
sthetizing doses of pentobarbital into the
her by permitting the mother to emerge from

sthesia and finally by reintroducing deep
sthesia in the mother by injecting additional
tobarbital These rapid and marked changes in

ologic state which themselves led to marked
rations in oxygen transfer to the fetus had the
ct of distorting the bars of the bar graphs il

rated in Fig 2 Thus when oxygen transfer to
fetus was rapidly reduced due to physiologic
ages in the mother during a 30 min period when

mother was breathing oxygen comparisons of
new fetal blood oxygen values with the values
ained during the preceding period when the

ther was breathing air leads to artifactual reduc
ns in the magnitudes of the differences in the PO_2
tal arterial blood under the two conditions of

s breathing This effect was so prominent early
ing the fourth hour as the mother awakened that
led to negative difference values when comparing

al PO_2 values with the mother breathing oxygen
d room air (see the reduced PO_2 values in blood
mples as taken from all sample sites of the fetus

arly during the fourth hour when the mother was
eathing oxygen compared to the preceding period

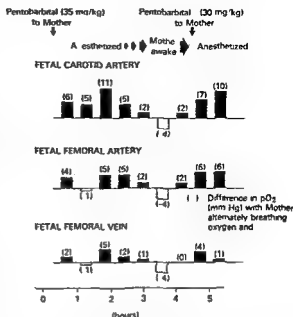


Fig 2 Bar graphs showing the magnitudes of the increases (or decreases) in PO_2 of blood sampled from the carotid and femoral arteries and the femoral vein of the term fetus of monkey 790H when she was breathing oxygen as compared to when she was breathing room air alternately for 30 min each over a 5 hour period. Negative values occurred when large physiologic changes took place in the mother or the fetus during a single 30-min interval when the mother was breathing oxygen leading to marked decreases in oxygen tension of fetal blood samples i.e. during that time when mother 790H awakened from anaesthesia when all samples of fetal blood showed marked decreases in oxygen tension

when she was breathing room air) Similar effects but of opposite sign were observed when marked alterations in oxygen transfer to the fetus occurred during periods of air breathing

Fetal subcutaneous tissue PO_2 was measured continuously using oxygen sensitive electrodes. When the mothers breathed 100% oxygen the oxygen tension of the fetal subcutaneous tissue increased by as much as 5 mmHg as illustrated in Fig 3a and b. These increases in tissue PO_2 were regularly delayed by about 50 sec after the mother started oxygen breathing. The tissues then required another 30-150 sec to reach their maximum oxygen values. Then after the mothers were abruptly shifted to air breathing the fetal tissue oxygen tension continued unchanged for another 40-60 sec before it began to decline and 100-180 sec more were required before the oxygen tension returned to its earlier value.

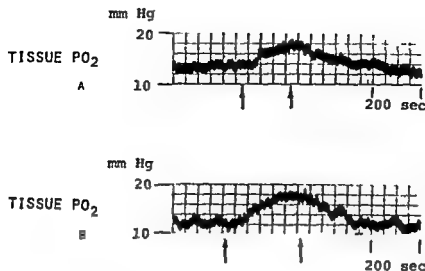


Fig 3 Changes in oxygen tension, cervical subcutaneous tissue of a full term monkey fetus following oxygen administration to the mother for 3 min 10 sec (a) and 5 min (b). The mother was breathing spontaneously during the entire period of recording.

DISCUSSION

When pregnant rhesus monkeys were switched from air to oxygen breathing comparisons of PO_2 values of samples of fetal blood as taken from all sites showed increases in oxygen tension in almost all instances. In those few occasions when such increases were not observed the physiologic state of the animals had changed sufficiently over the 30 min intervening between samples that the actual oxygen transfer to the fetus had diminished to an extent greater than the magnitude of the oxygen increment brought about by maternal oxygen breathing. In other unpublished studies fetuses sampled immediately before and after oxygen as administered to the mothers always showed increased oxygen values at all sites in all instances during the period of oxygen breathing. That oxygen administration to the mother leads to increased oxygenation of the fetus is also evident from the present results obtained with the oxygen sensitive electrode in fetal tissue.

The extent to which oxygen breathing by the mother increased oxygen values of the fetus varied with the physiologic state of the mother and the fetus. These increases were largest when the mothers were anaesthetized and least when they were nearly awake and in a stressed state on the operating table. Furthermore when the mothers were awake and the PO_2 values of all fetal blood samples were abnormally depressed, reinstitution of anaesthesia restored all of the fetal blood PO_2 values towards normal and increased the extent to which oxygen administration to the mother improved oxygenation of the fetus.

The increased oxygen transfer to the fetus brought about by oxygen breathing by the mother can be understood by considering the changes in maternal oxygen breathing produces in the composition of maternal arterial blood. When the PO_2 of maternal arterial blood falls within the range 95–100 mmHg as occurs when she breathes room air, hemoglobin contained within red blood cells is nearly fully saturated (>96%). When the arterial blood oxygen tension is then increased to 400 mmHg as occurs during oxygen breathing, the quantity of oxygen transported bound to hemoglobin is increased by only 0.8 vol% or by 4% of the total oxygen transported at normal oxygen tensions. At the same time the quantity of oxygen transported in physical solution which at normal oxygen tensions corresponds to 0.3 vol% or 11% of all oxygen carried increases to 1.9 vol% or 18% of all oxygen carried when the mother is breathing oxygen. Thus oxygen breathing by the mother increases the quantity of oxygen carried in the blood both bound to hemoglobin and especially in physical solution. The magnitude of the increase taken together is 2.4 vol% which means the mother's blood carries 12.2% more oxygen when she breathes oxygen than when she breathes room air. About 60–70% of this increased oxygen carrying capacity of blood brought about by oxygen breathing comes about as additional amount carried in physical solution. The effects of oxygen breathing on oxygen transport by blood have been more fully discussed by others (11).

The effectiveness of oxygen breathing in increasing the quantity of oxygen actually transported

nal blood is greatly increased in those circumstances when the oxygenation of the maternal blood is depressed below normal to start with. Such anemia is not uncommon and may occur as a consequence of maternal respiratory depression, a variety of pulmonary diseases, use of anesthetic agents or narcotics. High dose levels diminish maternal arterial blood oxygenation, depressing the mother's respiratory state while chronic asthma, emphysema or other pulmonary diseases decrease actual oxygen delivery to circulating maternal blood. When oxygenated air is administered under any of these conditions, the maternal arterial blood oxygen content is increased by amounts far greater than those presently described. Thus administration of oxygen to women in labor where respiration is depressed or pulmonary function is altered leads to far greater improvements in fetal oxygenation than did administration of oxygen in the present study. The oxygen tension of maternal arterial blood is maintained within normal range during the periods of air breathing.

Several factors in addition to oxygen content of maternal arterial blood affect net oxygen transport to the fetal blood stream. The blood pressure of the mother by determining the volume of blood through the intervillous space importantly influences the quantity of oxygen delivered to the fetus. Thus when the mean blood pressure of pregnant rhesus monkeys was rapidly reduced from a normal of 90-100 to 50 mmHg, the fetal arterial blood PO_2 decreased from 30 to 20 mmHg (13). The magnitude of such decreases in fetal arterial blood PO_2 brought about by lowering blood pressure in the mother may be sufficiently great as to cause fetal brain injury or death (3). Use of barbiturates at dose levels sufficient to cause significant reductions of maternal blood pressure invariably decreases oxygen tension in the fetal arterial blood. These latter effects of deep penitential anaesthesia can be reversed in every instance by restoring the blood pressure of the mother to normal.

Another factor which importantly influences oxygen transfer to the fetus is the state of tonus of the uterine blood vessels. The many factors which alter uterine vascular conductance have been reviewed in detail elsewhere (2) and will not be discussed further here. However, the important role played by uterine blood vessel tonus in determining oxy-

genation of the fetus is well illustrated in the present study. During the mother's emergence from anaesthesia, the oxygen tension of the maternal arterial blood and the maternal blood pressure were both well maintained or indeed sometimes elevated. Despite this, the supply of oxygen to the fetuses regularly declined to low levels as was demonstrated by the marked reductions of blood oxygen pressures at all sample sites. Under the same circumstances, the magnitudes of the increases in fetal blood PO_2 observed when oxygen was administered to the mothers also declined to low values indicating a decreased effectiveness of oxygen breathing in ameliorating asphyxia of the fetus under conditions of maternal stress. These decreases in fetal blood PO_2 at all sample sites due to diminished oxygen transfer to the fetus with stress of the mother can only be explained by assuming a decreased uterine artery blood flow and thereby a reduction in maternal blood flow through the intervillous space.

The oxygenation of the fetus was most profoundly depressed when the mothers were awake and restrained. Earlier studies have shown that stressing pregnant rhesus monkeys psychologically leads to diminished oxygenation of the fetuses (15). The decreases in fetal arterial blood PO_2 typically developed 50 sec after the psychologic stress was applied to the mothers and fetal oxygenation improved usually within minutes after stress was removed. These results indicate that maternal anxiety or fear can reduce oxygen transfer to the fetus. Such reduced oxygen transfer to the fetus has been explained as due to increased maternal sympathetic nervous system activity, constriction of blood vessels supplying the uterus and other visceral organs and reduced oxygen delivery to the fetuses. A similar action seems likely here to account for the marked reductions of fetal arterial blood PO_2 as the mothers roused from anaesthesia. That the oxygen tension of fetal blood samples inevitably augmented after these mothers were reanaesthetized supports this suggestion. The effects of catecholamine infusions in diminishing oxygenation of the fetus have already been described elsewhere (1, 8, 9, 16) and will not be further discussed here.

Significant decreases in PO_2 were observed in fetal blood samples in the present investigation during the second hour of study. These decreases appeared first in femoral and only later in carotid artery samples. The declines occurred at

when the mothers were still fully anaesthetized and their physiologic states stable. Though the mothers experienced some decreases in blood pressure at the same time these blood pressure decreases were not sufficient to lead to diminished oxygen values in fetal blood. Rather the reductions in fetal oxygenation more likely resulted from physiologic changes taking place in the fetuses themselves since the reductions did not appear simultaneously at all sample sites but rather appeared first in samples taken from the posterior circulation. The relations of these early diminutions in oxygen tension in fetal blood samples to the further decreases which develop as the hours pass remain unclear. However both the early and late declines need be distinguished from the superimposed changes in fetal oxygenation that occur as a result of changes in level of anaesthesia of the mothers. The basis for the apparently inexorable declines in fetal oxygenation which take place in rhesus monkeys during prolonged barbiturate anaesthesia remain entirely unknown. Whatever the basis for these changes may be their eventual understanding is critical to any efforts to find methods of treatment for intrauterine asphyxia of the fetus.

Fifty sec were required for the oxygen tension in fetal tissue to start to increase after the mothers began oxygen breathing. A similar lapse of time was required for the fetal tissue oxygen tension to begin to decline after the mothers were returned to air breathing. Such delays are entirely expected since the minimum time required for oxygen tension in fetal tissue to respond one way or another is that the time required for the maternal blood to circulate from the pulmonary alveolar capillaries to the left side of the heart and through the aorta and uterine blood vessels to the placental intervillous space. This circulation might be expected to take as long as 20–30 sec in the deeply anaesthetized monkey. An additional 20–30 sec is then required for fetal blood to absorb the added (or reduced) oxygen from the intervillous space and to transport it back from the placental villous capillaries through umbilical veins to the inferior vena cava and through the left side of the heart and ascending aorta to the subcutaneous tissues of the neck. The elapsed time of 50 sec for changes in fetal tissue oxygenation to appear after oxygen administration to the mother also compared closely with the 50 sec delay which are observed after the mothers are psychologically stressed and the fetuses begin to show decreases in

oxygenation (15). Though the mechanisms of response in these 2 circumstances differ in detail the times required for their response are similar. The short time required for fetal blood from the villous capillaries to the fetal neck agrees with the 10–20 sec minimum delay encountered between the beginning of a contraction and the first appearance of a decrease in the heart rate of the fetus (late deceleration) (11).

Oxygen electrodes as presently constructed are subject to considerable technical problems amongst these the consumption of oxygen (16). The degree to which this oxygen consumption affects the oxygen tension measured is difficult to estimate since the magnitudes of changes in oxygen tension induced in the vicinity of the electrode depends on its distance from nearby capillaries. In turn this distance varies from one electrode insertion to another and from one time to another during single insertion. Other sources of systematic error in oxygen measurement include differences in geometry of electrodes, in characteristics of membranes utilized and in diffusional and conformational properties of surrounding tissue.

The characteristics of local blood flow may also alter as a result of trauma during electrode insertion. If a hematoma forms around the electrode it may affect blood flow in the vicinity of the electrode membrane may also become coated with fibrin causing changes in PO_2 measurements. It is not possible to predict the magnitudes of these various errors; frequent calibrations of electrodes are necessary. Measurements carried out with electrodes left in position for long periods of time are therefore questionable. In addition, sources of error related to electrode construction and alterations induced in tissue by electrode insertion have been discussed by Grunewald (6).

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1**Clipsapplikator Hulka****2****Ringapplikator Lay****3****Bipolar griptång****4****Endotherm griptång, teflonbelagd****WOLF**
laparoskop Palmer**NYHET****LUMINA-SL-optik**
Ljusare, större upplösningsskärpa. Raka laparoskopoptiker, 10, 7 och 5 mm**W**
WISEX

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QUANTIFICATION OF FETAL HEART RATE VARIABILITY IN RELATION TO OXYGENATION IN THE SHEEP FETUS

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Beat-to-beat variability has been suggested as a
ve indication of fetal well-being. The suggestion
ates from case reports of observed decrease in vari-
ty preceding clinical signs of fetal asphyxia and fetal
1. Against this background a study was performed to
ate possible changes in heart rate variability in rela-
to changes in fetal oxygenation. Chloralose
thetized sheep were used. Short term hypoxia was
uced without acidosis. The ECGs were recorded on
atic tape and later analysed by scrutinizing each QRS
plex prior to the trigger of a rate meter. The differen-
index (DI) described by Yeh and co-workers was
en because it is easily computed and reflects the
ability of coefficient of variation for two successive
intervals. In contrast to the generally held view of a
ressive diminution of variability during the develop-
t of asphyxia, the variability was found to increase
dial with a decrease in fetal arterial PO_2 determined on
d samples withdrawn at intervals and ranging between
nd 10 mmHg. The correlation between variability and
in the range below 8-10 mmHg is still to be deter-
ed.

INTRODUCTION

e diagnosis of impending hypoxia is usually made
er interpretation of the simultaneous recording
heart rate and intra uterine pressure. Thereby
idycardic episodes following uterine contrac-
ns at defined intervals are considered to reflect
oxygen availability and to represent a hazard
s situation for the fetus. Bradycardia as a conse-
ence of hypoxia is however a late sign (1).
Therefore other parameters are sought which
ight change characteristically within higher ranges
oxygen tension. The differences in duration be-
ween consecutive heart cycles have been proposed

as a suitable parameter for this purpose (1, 6, 7, 8, 9, 14).

Hammacher (5) introduced the concept of
oscillation amplitude as a means of expressing heart
rate variability in quantitative terms. The alteration
in argument following the plotting of consecutive
heart cycle lengths against each other in a two-
dimensional system according to de Hahn (3) and
the differential index of Yeh (15) serve the same
purpose. All these three indices describe short
term variability. The generally accepted view is
that this variability is high when the fetus is in a
relatively undisturbed condition but decreases
when the oxygen supply is reduced.

The present study was undertaken to evaluate
possible changes in heart rate variability related to
changes in fetal oxygenation. An animal experimen-
tal model was used in order to make possible re-
cording of fetal ECG during graded hypoxia without
the interference of severe acidosis. An additional
purpose was to establish the relationships between
fetal heart rate variability during hypoxia and other
cardiovascular parameters such as arterial blood
pressure, fetal ECG pattern and cardiac contrac-
tility.

The ultimate goal of the study would be to ascer-
tain which variables contain most information about
impending oxygen lack to the fetus.

MATERIAL AND METHODS

The experiments were conducted on 8 ewes of mixed
breed with 11 fetuses. The gestational ages ranged from
123 to 140 days (term 147 days). The gestation was either



1

Clipsapplikator Hulka



2

Ringapplikator Lay



3

Bipolar griptång



4

Endotherm griptång, teflonbelagd



WOLF
laparoskop Palmer



NYHET

LUMINA SL-optik
Ljusare, större upp-
lösningsförmåga Ra-
ka laparoskopoptiker,
10, 7 och 5 mm

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response to hypoxia. Thus there are no signs of failing myocardial function associated with early increase in fetal heart rate variability and the appearance of ECG changes. In case A a decrease in D I was noted after 30 minutes of hypoxia ($aPO_2=6$ pH=7.27). The values were obtained at the beginning of the functional failure of the cardiovascular system as demonstrated by a fall in maternal arterial pressure, FHR, left ventricular myocardial contractility and maximal changes in the ECG.

DISCUSSION

In the endeavour to detect developing fetal hypoxia interest has been focused on the variability of fetal heart rate. Originally the variability was discussed on the basis of phonocardiograms (5) but subsequently ECG signals were used to trigger heart rate meters. Along with development in electronics true beat to-beat recordings were made available and the synonymous description has been instantaneous FHR recording or the use of non averaging systems.

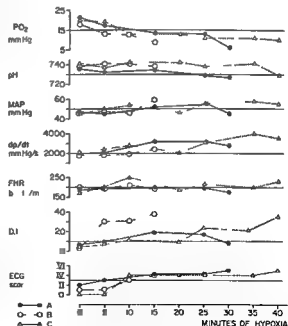


Fig 3 The relationship between aPO_2 , pH and various cardiovascular parameters: mean arterial pressure (MAP), myocardial contractility (max dp/dt), fetal heart rate (FHR), heart rate variability (D I) and ECG score during continuous hypoxia in three mature sheep fetuses.

RESULTS

Correlation between arterial PO_2 determined from good samples from the right brachial artery of fetuses and the corresponding D I value is visualized in the scattergram (Fig 1). There is a lack of information regarding the relationship between aPO_2 and D I in the range below $aPO_2=8$ mmHg. The regression equation is therefore based on the observations between 10 mmHg and 26 mmHg. The regression of D I on aPO_2 has the equation $D I = 3-1.7x$. The correlation coefficient is -0.78 , $p < 0.001$. The 95% confidence limit of the regression line is minimum ± 2 mmHg.

The relation between pH and D I is shown in Fig 2. It is observed that the majority of the observations are distributed between pH 7.20 and pH 7.42. Only 8% of the observations fall below 7.17. Observations within the range 7.20-7.42 are only distributed in different cardiovascular functions: arterial pressure (MAP), left ventricular myocardial contractility (max dp/dt), fetal heart rate (FHR), fetal heart rate variability (D I) and pressure changes in the S T interval of the fetal ECG are plotted together with aPO_2 and pH against time in Fig 3. The values in the figure were obtained from three fetuses subjected to varying degrees of hypoxia without acidosis.

The general tendency seen in the cardiovascular response to hypoxia is some increase in MAP and in max dp/dt value might indicate an increase in myocardial contractility as a

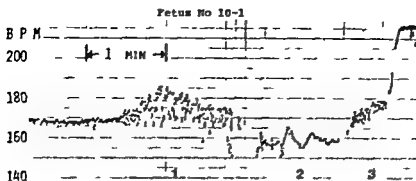


Fig 4 Change in variability during progressive development of hypoxia. 1 Marked variability induced variability associated with a PO_2 value of 6 mmHg. 2 Reconstitution.

In the usual FHR recording the beat to-beat variations are difficult to quantitate Hammacher (6) has introduced the term oscillation amplitude and subdivided the oscillation range in 3-4 subgroups de Hahn (3) has described the short term variations from vector analyses of consecutive heart cycle lengths while Yeh (15) has described the differential index used in this study. In the DI the consecutive cycle length differences are related to the actual mean level of two heart cycles each d is proportional to the coefficient of variation for consecutive heart cycle lengths. Similar indices can be constructed in several modifications but it is likely that the information gained in this respect would not be much improved.

The general concept regarding the heart rate variability is that its index value is large in the situation of fetal well-being and decreases in proportion to deterioration until a "silent pattern" is seen (1). It is therefore contrary to expectation to find that under the circumstances of this study the DI values increase along with the diminution of PO_2 until a PO_2 level of 10 mmHg is reached. It remains to be seen whether or not the index values are correlated to PO_2 in the range below 8-10 mmHg. A sufficient number of observations within this low range has not hitherto been obtained. The clinically observed situations of "silent pattern" correlated to poor fetal outcome might represent a PO_2 value in an even lower range.

When comparing other variables reflecting the status of the fetus it appears as if the increase in the DI is associated with a still preserved circulatory function as judged from the behaviour of MAP, FHR and max dp/dt in the cases analysed in Fig 3. The increase in DI seems to be associated with the anaerobic utilization of myocardial glycogen

since alterations in the S-T interval of the fetal ECG were recorded at the same time. These changes have in previous studies been shown as a sign of myocardial glycolysis during hypoxic stress (12, 13).

In this study the ultimate decrease in fetal heart rate variability was induced around 8-10 mmHg. Acidosis was not present however and drugs had not been administered. The level of oxygen pressure associated with the decrease in variability during clinical situations needs to be elucidated further.

The present study has some drawbacks: the use of anesthetized animals even if chloralose is stated to be the ideal anesthetic agent for study of cardiovascular reflexes (4) and for continuous oxygen tension determination. A preliminary report by Dalton and co-workers (16) points in the same direction however (2). The authors found that hypoxemia in chronic anesthetized sheep fetuses caused a large sustained increase in heart rate variability measured either as the S-D of the heart period or as the beat to-beat difference.

In the future elucidation of the problem a technique must be developed enabling one to correlate actual heart cycle length with the simultaneous PO_2 value derived from continuous recording of PO_2 means of an indwelling arterial or a transcutaneously applied oxygen electrode. Various methods and electrode placements must be tested in order to investigate a possible time difference between the actual PO_2 and the change in heart cycle length. It would have been of considerable interest for example to determine the temporal relationship between the gradual development of hypoxia and the induced change in variability in the fetal heart rate.

r 10-1 The dynamics of the progressive es in beat-to-beat variability along with al deepening hypoxia in this case is shown in

CONCLUSIONS

1 acidotic exteriorized term sheep fetuses of close anesthetized mothers the variability of heart rate expressed as differential index was 2 ely proportional to fetal arterial PO_2 within 3 ange of 10-26 mmHg This observation is in 4 addition to the generally held view and needs 5 boration in further extended studies using a 6 y of species and including determination of 7 polic parameters

ACKNOWLEDGEMENTS

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STILBESTROL ADMINISTRATION IN THE PUERPERIUM AND ITS EFFECT ON THE PROLACTIN EXCRETION OF NON LACTATING PATIENTS

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act The relationship between stilbestrol treatment placebo treatment was studied in 2 groups of non ng patients. Plasma prolactin levels were evaluated in 2 groups on day 1 3 and 7 of the treatment by a immunoassay technique with the double antibody od using Sero-n hPRL kit. Our results show that ctin values increased significantly in the stilbestrol 3 and decreased in the placebo group. It seems the main reason for the drying up of milk is the of stimulation of the nipples which after a relatively tant time of 5-10 days will lead to decreased prolactin s. Stilbestrol treatment is not effective in drying up milk more rapidly in patients who do not want to.

When patients have symptoms a combination of pessa and breast support usually brings relief within 24 hours.

maternal ability to secrete milk depends on quate proliferation of mammary glandular tissue ducts during pregnancy. This proliferation is ulated by the increase in estrogen, proges me and lactogen. After parturition prolactin is an important and major part among other mones in initiating milk secretion by its ogenic activity and in maintaining milk produc t by its galactopoietic action (1 2).

Unlike other pituitary hormones which are ce ted following stimulation from the hypo- lamus prolactin is inhibited by the influence of Prolactin Inhibitor Factor (PIF). Thus therefore ans that the release of prolactin is continuously pt in check and that substances which decrease

PIF levels act by the withdrawal of inhibition uch results in increased prolactin secretion. Vari s investigators have also shown that the suckling let leads to an increased release of prolactin.

Thus the infant by suckling activates the nerve receptors at the base of the nipple and causes the release of prolactin (3). It could be assumed that this reflex is brought about by oxytocin release but this does not appear to be the sole reason as it can be demonstrated that oxytocin does not cause prolactin release in tissue from the rat hypophysis cultured in vitro (4).

When the mother is not nursing suppression of lactation is of importance. Although the cessation of the nipple stimulation effect and suckling are of major importance many therapeutic methods have been suggested to shorten and ease the process of "drying up the milk" (5 6 7). Most of these treatments if not all are known to be of questionable efficiency.

The development of a reliable and specific radioimmunoassay for human plasma prolactin enabled evaluation of prolactin levels from birth to adult life and especially the values in plasma during the puerperium in nursing and non-nursing mothers (8). It seems to be one of the major hormones controlling milk formation.

This study was undertaken to evaluate various drugs currently in use for the suppression of lactation particularly stilbestrol by studying the plasma prolactin levels in non nursing patients.

MATERIAL AND METHODS

A total of 18 patients who for various reasons requested to bottle-feed their offspring were considered for this study. Two of them were primiparas the 16 others were

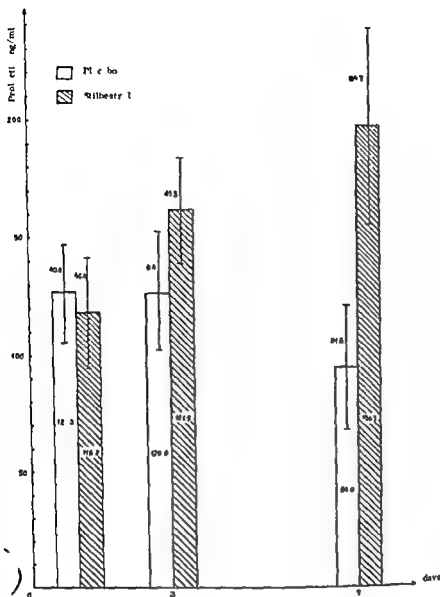


Fig 1 Prolactin levels in nursing mothers treated with placebo or stilbestrol given in ng/ml \pm S.D.

multiparas with 2 to 4 deliveries including the present one. They all had normal spontaneous deliveries at term.

These patients were divided into 2 groups: the first group consisting of 17 patients was treated with stilbestrol given orally 3 tablets a day of 5 mg each for 5 consecutive days starting on day one following delivery. For the purpose of this study day 1 was calculated as 18 to 24 hours following time of delivery.

The 2nd group consisting of 9 other patients were given a placebo consisting of a multivitamin tablet presented exactly like the stilbestrol tablet in shape, color and size. It was given similarly to the stilbestrol 3 times a day for 5 consecutive days. The nurses in charge of drug administration were not informed which of the patients were receiving the stilbestrol tablets or the placebo, thus eliminating bias in reporting complaints by the patients about breast tenderness or engorgement, milk secretion and fever. Recording of these complaints was carried out by

the nurse and/or by the resident in charge of the ward. Both nurses and resident were not informed of the treatment. The follow-up was continued for a period of 14 days from the time of delivery and patients were therefore asked to come back for a check-up. Both groups were advised to support breasts in a tight and well-fitting brassiere every night.

Five ml of blood were withdrawn from these patients and collected on heparin so as to collect the supernatant plasma following a 15 min centrifugation at 2000 rpm. Plasma was then stored in deep freeze.

Three samples were collected: one day following delivery on day 3 of the treatment and on day 7 following delivery. Collections of blood were carried out at the same time on each occasion.

Plasma prolactin values were estimated by a

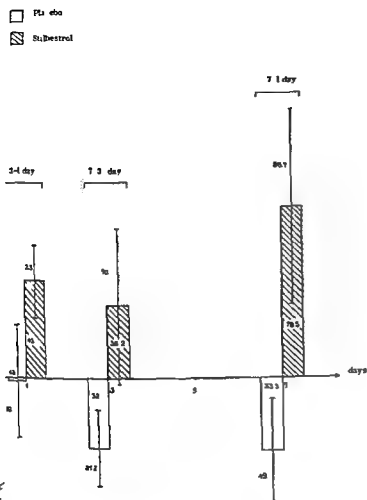


Fig 2 Difference in prolactin levels in non-nursing mothers treated with placebo or stilbestrol (values are given in ng/ml \pm S.D.)

assay with the double antibody method. This assay uses the Serono technique and their hPRL kit. The sensitivity of this method is about 5 ng/ml.

RESULTS

Plasma prolactin values in these two groups of non-nursing patients treated randomly by stilbestrol or by a placebo are schematically presented in Fig 2 and expressed in ng/ml \pm S.D.

When the treatment starts with stilbestrol the mean prolactin of day one following delivery was 118.2 ng/ml with a standard deviation of ± 46.4 . On day 3 following delivery 126.0 ng/ml ± 48.4 and on day 7 following delivery 94.0 ng/ml ± 51.8 .

In the placebo group on day one following delivery

ery prolactin was found to be 127.3 ng/ml ± 40.0 on day 3 126.0 ng/ml ± 48.4 and on day 7 following delivery 94.0 ng/ml ± 51.8 .

Statistical analysis showed a *p* value of less than 0.4 for day one between the placebo users and the stilbestrol treated patients, *p* less than 0.1 on day 3 and a highly significant difference on day 7 with *p* value of less than 0.05.

In fact the results showed a significant rise of plasma prolactin levels from day 1 to 7 in the group treated with stilbestrol from 118.2 ng/ml ± 46.4 to 196.7 ng/ml ± 84.7 . In the group treated with placebo prolactin varied from 127.3 ng/ml ± 40.0 on day one to 94.0 ng/ml ± 51.8 on day 7 following delivery which is not significantly different.

Fig 2 expresses the difference in plasma pro-

lactin levels in ng/ml in these 2 groups of non nursing mothers. Differences were calculated between day 3 and 1 of the treatment and 7 and 3 of the treatment and 7 and 1 of the treatment. Results were as follows: a negative difference of 1.3 ng/ml for the placebo group compared to a positive difference of 43.3 ng/ml in the stilbestrol group between day 3 and 1 with a p less than 0.025 which is highly significant. Difference between day 7 and 3 was negative in the placebo group to 32.0 ng/ml and a positive difference of 35.2 ng/ml for the stilbestrol group with a p less than 0.0125 and again a very significant difference between day 7 and 1 with a negative difference for the placebo group of 33.3 ng/ml to a positive difference of 78.5 ng/ml for the stilbestrol group and a p value of less than 0.025.

As for the clinical results only five of the patients treated with stilbestrol complained of side-effects as compared to 9 patients in the placebo group. However the side effects in the stilbestrol patients were expressed as more severe in intensity especially engorgement, pain and the discharge of milk compared with slight engorgement in the placebo group. No fever was reported in either group.

It may be of interest to note that these side effects appeared earlier in the placebo-treated patients than in the group treated with the stilbestrol. In the placebo group they started on day 3 while in the stilbestrol group on day 5 to 10.

Most of the side effects disappeared 24 to 48 hours after their appearance in the placebo group and 24 hours to 3 days following their appearance in the stilbestrol group.

DISCUSSION

A relatively large group of new mothers do not nurse their babies for various reasons (9). These post partum patients seek constant advice and treatment for the purpose of milk suppression. Stilbestrol is along with various other estrogen preparations the drug of choice used for suppressing lactation in many maternity centers. These drugs are prescribed and are considered efficient based mainly on clinical observations and reports in the literature (10, 11) but in most of them they were carried out with no definite control and with a relatively short follow up. Moreover in one clinical study (12) no differences were found in the symp-

toms of 500 non nursing mothers treated with or without stilbestrol.

Although the hormonal basis of milk production is only partially understood it seems that prolactin is of primary importance and therefore can be of use in evaluating treatments used for suppression of lactation.

Our results stress the fact that plasma prolactin values far from decreasing are actually increased significantly when patients are treated with stilbestrol and when compared with patients who received placebo treatment. It seems that the reason for the drying up of milk is the lack of stimulation of the nipple of the breast which has its effect after a relatively constant time interval from 5 to 10 days. Time is the factor responsible for the decreased prolactin level in the group of patients treated with the placebo drug. The fact that stilbestrol increases the plasma prolactin level can be partially explained by a feedback mechanism which stilbestrol reduces the hypothalamic secretion of prolactin inhibitor factor.

From the clinical point of view these results of high excretion of prolactin may explain the fact that patients treated with stilbestrol will complain of their symptoms later than in the group treated with a placebo. In the same manner it seems that the symptoms and in particular breast engorgement, tenderness and milk discharge were more often felt by the stilbestrol treated patients when it was up to continued after the period reported in this study.

From these observations one can conclude that stilbestrol treatment or other drugs with anti-estrogenic effect are not efficient for the treatment of stopping milk secretion in patients who do not want to nurse their babies.

In cases complaining of clinical symptoms a combination of analgesia and breast support and other local treatments usually bring relief within 24 to 48 hours. It seems that the time following non stimulation of the nipple is among the main factors responsible for the suppression of lactation.

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CHANGES OF BONE MINERAL CONTENT DURING PREGNANCY AND LACTATION

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act In order to study changes in the calcium depots of the body during pregnancy and lactation bone mineral measurements were performed on fourteen pregnant women and eighteen mothers post partum by X ray roenthenography. Pregnant women exhibited a loss in bone mineral in the total body but not in cortical bone when measured during late first or early second trimester and again one week post partum. Lactating women who nursed less than three months lost mineral during the first three months and then regained it while those who nursed for more than three months had no losses during six months post partum.

The high incidence of fracture in postmenopausal women due to osteoporosis constitutes a social and a medical problem. It is possible that pregnancy can to some extent prevent future postmenopausal osteoporosis as retrospective investigations using roenthenographic absorption techniques indicate an increase in bone mineral with parity (4, 9, 10). Moreover, osteoporosis seems to be overrepresented among women with spinal osteoporosis (11). The purpose of the investigation reported here was to determine whether pregnancy and lactation influence the bone mineral content.

MATERIAL AND METHODS

Women who in an early stage of pregnancy attended an antenatal clinic were asked to participate in an investigation of bone mineral changes during pregnancy. Fourteen women completed the study. By means of X ray roenthenography the regional bone mineral content was determined in two sites of the forearm. The measurements were performed in the late first or the early second trimester and repeated one week after delivery. The measuring sites were one and eight cm proximal to the radiocarpal joint representing trabecular and cortical bone respectively.

The postpartum period was the subject of a separate investigation. Women in a maternity ward were asked to participate in an investigation of bone mineral changes during lactation. Eighteen volunteers completed the study. Measurements were performed one week after delivery and repeated 3 and 6 months postpartum.

Measurements were also carried out on nine non-pregnant and non lactating controls during the same period. The post partum women and the controls were questioned about their milk-drinking habits, hormone treatment and previous ability to breast feed. The measuring sites were situated in the forearm 1 and 8 cm proximally to the radiocarpal joint, the femur 20 cm proximally to the basis patellae and in tuber calcanei.

The bone mineral content was determined by X ray roenthenography (5, 6). In this method the different

Table 1 Age, previous pregnancies, previous lactation and exposure to contraceptive hormones during the study period

	No of patients	Age in years		Previous pregnancies (Mean)	Previous lactation (No.)	Exposure to hormones (No.)
		Mean	S.D.			
Controls	9	26	4.7	0.2	1	2
Pregnant	14	28	4.7	0.9	9	0
Lactating	18	29	3.1	0.7	11	2

Table II *Bone mineral changes during pregnancy*

	Mean (%)	S D	P
Radius+ulna shaft	+2.0	5.5	>0.05
Radius+ulna distal	-4.2	7.0	<0.05
Mean of both sites	-1.1	5.2	>0.05

skeletal parts are positioned in a fluoroscope and scanned with a beam from an X ray tube. The beam comprises two wavelengths by means of which the attenuation in the soft tissues surrounding the bone can be compensated for. The measuring sites and the measuring precision have been described by Dalén & Jacobson (3).

RESULTS

Details of age, previous pregnancies, previous lactation and exposure to contraceptive hormones during the follow up period are given in Table I.

In the pregnant group the mineral content decreased in the distal measuring site containing trabecular bone (Table II). There was no significant change in the diaphyses of the forearm containing cortical bone.

In the postpartum group no systematic difference was found between mineral changes in trabecular or cortical bone. Nor was there a difference between weightbearing and non weightbearing parts of the skeleton. As seen in Table III where the mean deviation of four sites for each individual is given, mothers lactating for less than three months at first post and then regained bone mineral as compared with controls. Mothers lactating for more than 3 months did not differ from the controls (Table IV).

Two subjects who ceased breast feeding at an early stage took contraceptive pills (Lynestrenol) during the post partum period. The bone mineral changes in these women did not deviate from those of the others.

No correlation could be detected between changes in mineral content and age, number of previous pregnancies or milk consumption.

DISCUSSION

During reproduction changes of hormonal secretion and calcium metabolism influence the mineral content of the skeleton in some animals. In avian preovulatory increase of estrogen induces growth of endosteal bone which serves as a calcium reservoir for the eggshells (7). In mammals estrogen can stimulate bone growth but this does not occur during normal reproduction (8). The bone mineral changes during lactation have been studied for instance in cattle. A low intake of calcium leads to reversible mineral losses but during normal conditions there is a small increase in bone mineral content during both pregnancy and lactation (1).

In humans retrospective studies by photodensitometry techniques indicate an increase of bone mass with parity (4, 9, 10). Few prospective studies by this technique have been carried out. Christiansen et al. (2) found a constant mineral content of the forearm during pregnancy in thirteen women and Atkinson & West (1) recorded a loss of about 2% in the femur diaphyses of ten lactating women during a period of hundred days.

The current view on the mineral changes in women seems to be that mineral is stored during pregnancy and lost during lactation (4).

The present study showed a small but significant loss of mineral from trabecular bone during pregnancy and hence the results do not favour the hypothesis that pregnancy leads to an increase in bone mass. Trabecular bone constitutes about twenty per cent of the total bone mass but is metabolically more active and reflects changes of the calcium metabolism earlier than cortical bone.

The finding that mothers lactating for only a short

Table III *Bone mineral changes in women lactating for less than three months as compared with controls*

	No	0-3 months			3-6 months		
		Mean of four sites (%)	S D	P	Mean of four sites (%)	S D	P
Lactating	7	-2.6	3.2	>0.05	+9.1	5.0	<0.05
Controls	9	+1.8	4.8	>0.05	+1.7	6.4	>0.05
Difference		-4.4		<0.05	+7.4		<0.05

IV Bone mineral changes in women lactating for more than three months as compared with controls

	No	0-3 months			3-6 months		
		Mean of four sites (%)	S D	P	Mean of four sites (%)	S D	P
lactating	11	+1.7	3.7	>0.05	+4.0	3.0	<0.05
controls	9	+1.8	4.8	>0.05	+1.7	6.4	>0.05
difference		-0.1		>0.05	+2.3		>0.05

lost more bone mineral than mothers lactating for a longer period was unexpected. It is possible that some mothers cannot adapt themselves to the increased load on the calcium metabolism and therefore not able to breast feed for a long time. It is also possible that some factors may influence both the mineral content of the skeleton and the ability to breast feed for instance stress or hormonal disturbances. However, the losses were considerable and half a year after delivery there was a difference in mineral content between controls and women post partum.

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RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND SODIUM IN NORMAL PREGNANCY A LONGITUDINAL STUDY

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Abstract. Plasma renin activity, the concentration of angiotensin I and the urinary excretion of aldosterone and sodium were studied longitudinally in 10 healthy primiparae from the 10th week of pregnancy monthly until the 7th puerperal day. Plasma renin activity and angiotensin I levels were significantly elevated throughout pregnancy and showed maximal mean values at the 10th week, gradually returning to the level of nonpregnant women 7 days after delivery. The daily urinary excretion of aldosterone was also increased throughout pregnancy. The mean values showed an increasing trend until 34th week. They returned to nonpregnant levels 7 days after delivery. There were no significant changes in the urinary excretion of sodium during pregnancy but the mean value was lower than in the nonpregnant control group. There was a significant positive correlation between urinary aldosterone and sodium values, while an almost significant negative correlation existed between urinary aldosterone and plasma renin activity, suggesting the priority of aldosterone in the regulation of aldosterone secretion during pregnancy. No correlation was found between plasma renin activity and urinary sodium excretion values.

The increase of plasma renin activity and plasma angiotensin II concentration during pregnancy is well documented (4, 11, 21). The level of plasma renin substrate increases mainly due to the effect of oestrogens (5) and progesterone on the liver (22), but plasma renin concentration has also been shown to increase (4, 29). Plasma aldosterone concentration, aldosterone secretion rate and the urinary excretion of aldosterone are known to rise during pregnancy (1, 4, 30) and near term the excretion may be as much as ten times that of nonpregnant women (7). As a result of the increase of glomerular filtration during pregnancy the filtration of sodium is also increased about 50% (10) but due to increased reabsorption there is a positive sodium balance during pregnancy (4).

In the nonpregnant state there is a clear correlation and interaction between components of the renin-angiotensin-aldosterone system and this system has an intimate correlation with the body sodium-water balance as well (3). However, there are few and conflicting reports about these interrelationships during pregnancy. Also the regulation of this system during pregnancy is quite unclear (29). To solve these problems more systematically we studied the profiles and correlations of plasma renin activity (PRA), angiotensin I (A I) and urinary excretion of aldosterone (U Ald) and sodium (U Sod) longitudinally during and after normal pregnancy.

SUBJECTS AND METHODS

Ten healthy primiparae with a mean age of 24.6 ± 0.5 (S.E.M.) years (range 21-27 years), prepregnant weight 51.9 ± 1.0 (range 49-59 kg) and with a mean weight gain of 12.2 ± 0.8 kg (range 6-14 kg) participated in the study from the 10th week of pregnancy until term. The blood and urine samples were collected every 4th week with the last samples taken in the 38th week of pregnancy. Samples were also collected in hospital on the 7th puerperal day. 27 nonpregnant healthy women with comparable age and weight formed a control group. No dietary restrictions were required. None of the subjects had treatment with diuretics.

The blood samples for the PRA and A I determinations were taken at noon after two-hour fast and the maintenance of upright position for 4 to 5 hours. The 5 ml sample was always withdrawn into a chilled EDTA tube in icewater. Plasma was separated in 10 min by a refrigerated centrifuge at $+4^{\circ}\text{C}$ and stored at -20°C until analysis. The concentration of angiotensin I in the chilled plasma sample was determined by radioimmunoassay. EDTA, 8-hydroxyquinoline and dimercaprol were used for inhibition of converting enzyme and angiotensinases. The specific angiotensin I antiserum and standard and labelled angiotensin I were obtained from New England Nuclear.

Table 1 The mean (\pm S.E.M.) values of plasma renin activity (PRA) angiotensin I (A I) and aldosterone (dU Ald) and sodium (dU Sod) excretions in the 10th 34th and 38th week of pregnancy (n=10) on the 7th puerperal day and in the nonpregnant control group (n=27)

	Pregnancy					Non pregnant
	10th week	34th week	38th week	Puerperal		
PRA (ng/ml/h)	27.9 \pm 9.9	17.6 \pm 2.7	11.8 \pm 3.2	1.1 \pm 0.2		2.4 \pm 0.3
A I (ng/ml)	1.2 \pm 0.2	1.0 \pm 0.2	0.9 \pm 0.2	0.6 \pm 0.1		0.4 \pm 0.1
dU Ald (ug/day)	20.8 \pm 4.0	68.6 \pm 16.8	51.7 \pm 7.1	3.8 \pm 0.9		3.7 \pm 0.3
dU Sod (mmol/day)	114.0 \pm 11.0	108.0 \pm 15.0	138.0 \pm 15.0	138.0 \pm 15.0		146.0 \pm 1.0

For the PRA the angiotensin I generated by plasma renin in one hour at $+37^{\circ}\text{C}$ at pH 6.0 maleate buffered was determined by radioimmunoassay as above. Retaining the plasma samples in ice water until half an hour before centrifugation had no effect either on the A I or the PRA levels in plasma samples.

For the determinations of urinary aldosterone and sodium excretion the daily urine was collected in 2 parts between 7 am and 7 pm and between 7 pm and 7 am. Free and acid labile glucuronized aldosterone in the urine were determined by radioimmunoassay using first the method of Bayard et al. (2) for hydrolysis and extraction and for chromatographic purification the method of Ekins et al. (7). Urinary sodium was determined by atomic absorption spectrophotometer (Varian Tectron Model 1100). The intra assay precision (\pm S.D.) of 10 samples in the same series was 0.38 for PRA at the level of 4.5 ng/ml/h, 0.1 for A I at the level of 1.0 ng/ml and 5.5 for U Ald at the level of 65.7 $\mu\text{g}/\text{a day}$. The inter assay precision (\pm S.D.) for the PRA in 17 different series was 0.15 at the level of 1.14 ng/ml/h.

RESULTS

The noon PRA during the pregnancy is presented in Fig. 1. The mean PRA in the 10th week is higher ($p<0.05$) than the mean PRA in the 38th week (Table 1). All PRA values during pregnancy are higher than the values on the 7th puerperal day ($p<0.01$). The mean puerperal PRA is however lower ($p<0.05$) than in the nonpregnant control group.

The profile of angiotensin I levels (Fig. 1) during pregnancy is similar to that of PRA. The highest mean value was again found in the 10th–14th weeks of pregnancy and a decreasing trend was found afterwards (Table 1). The mean puerperal level of angiotensin I was lower ($p<0.05$) than during pregnancy. In the control group the level of angiotensin I was comparable with the puerperal level.

The day/night and 24 hour aldosterone excretions during pregnancy are presented in Fig. 2. By the 10th week the mean urinary aldosterone excre-

tion is already higher ($p<0.001$) than the puerperal or normal control values. In advancing pregnancy the excretion of aldosterone increases until the 34th week and this increase from the 10th to 34th week is significant ($p<0.05$). The mean urinary aldosterone excretion at all stages of pregnancy are higher ($p<0.001$) than the nonpregnant values and the 34th week excretion is about 10 times as much as the nonpregnant state. All values of the day/night excretion of aldosterone during pregnancy are higher ($p<0.001$) than the night values.

The urinary excretion of sodium remained unchanged during pregnancy (Fig. 3). It was similar to the puerperal excretion. During the 1st pregnancy the urinary excretion of sodium was higher than in the control group ($p<0.01$).

The correlation between the PRA and angiotensin I values is significant ($p<0.001$) but a significant relation was found between the renin-angiotensin system and sodium excretion. There is no significant correlation between individual values of aldosterone excretion and PRA or angiotensin I.

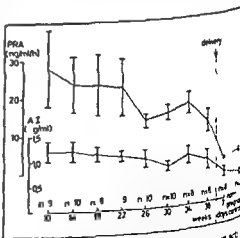


Fig. 1 Mean levels (\pm S.E.M.) of plasma renin activity (PRA) and angiotensin I (A I) during pregnancy.

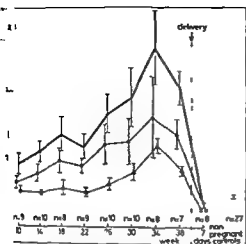


Fig 2 Mean levels (\pm S.E.M.) of urinary aldosterone excretion (U Ald) of 12 daytime hours (open circles) 12 nighttime hours (open triangles) and 24 hours (thick line) during pregnancy

There is significant negative correlation between mean values of u Ald and PRA until the 10th week of pregnancy ($p < 0.05$). There is a positive correlation between the urinary excretion of aldosterone and sodium ($p < 0.001$). The correlation coefficients and the significances are presented in Table II.

DISCUSSION

In this study the PRA levels are higher during pregnancy than in non-pregnant women and thus accord with previous findings (4). The highest values were found in the 10th week of pregnancy and there was a significant decrease towards the end of pregnancy. This is in agreement with the findings of Weir et al (30), Robertson et al (19), Tapia et al (23) and Gordon et al (11). On the other hand a previous general opinion (4) is that the PRA level rises (9) or remains unchanged (21) in the course of pregnancy until its termination. This reflects the difficulties in interpreting and comparing the PRA results found with different methods in different physical conditions. In this study all the individual PRA values during pregnancy were above and all the puerperal values below the upper normal limit of 3.7 ng/ml/h as measured by our method.

The level of angiotensin I was also higher in pregnancy than in non-pregnant women, a finding comparable with the results of Tapia et al (24) during

oral contraceptive therapy. The rise of angiotensin I level is probably a reflection of the rise in PRA since A I is an almost inactive intermediate in the formation of the physiologically very potent angiotensin II. The level of angiotensin II is also known to increase during pregnancy (11, 15, 29) but the profile of its secretion and its interactions are not known. The profile of A I levels during pregnancy is similar to the profile of PRA and the levels during pregnancy are in a significant positive correlation.

For aldosterone secretion during pregnancy there are different profiles reported. Weir et al (30) found maximal plasma aldosterone values in the 16th week of pregnancy while in other studies the excretion of aldosterone glucuronide (27) and tetrahydroaldosterone (17) have been shown to increase progressively up to the term which is in agreement with the findings of this study. Previously it was shown that the overall metabolic clearance rate of aldosterone is not greatly altered in pregnancy (30). The rise of urinary aldosterone excretion in this study from the 10th to 34th week of pregnancy is significant. Thereafter the excretion decreases slightly until term and reaches the non-pregnant excretion level in 7 days after delivery.

In general the rise of aldosterone secretion plays an important role in sodium balance during pregnancy. The blood flow in the kidneys increases in early pregnancy (20) and the filtration of sodium increases in parallel. If there were no antagonistic effect to the natriuresis the mother would lose all

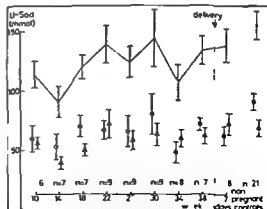


Fig 3 Mean levels (\pm S.E.M.) of urinary sodium excretion (U Sod) of 12 daytime hours (open circles) 12 nighttime hours (open triangles) and 24 hours (thick line) during pregnancy

HYSTERECTOMY IN CENTRAL RECURRENCE OF CARCINOMA OF THE UTERINE CERVIX

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Abstract Central recurrence was recorded during 1963-1972 in 136 patients (14%) out of a group of 977 cases in radiation therapy for carcinoma of the uterine cervix (Stages I and II). Fifty of these patients (37%) showed indications for surgery. In 11 of them only explorative laparotomy was performed. Radical hysterectomy was performed in 14 cases, combined vaginal and abdominal extrafascial hysterectomy in 13 cases and ordinary extrafascial hysterectomy was chosen in 11 cases. The cell morphology in the squamous-cell cancers classified according to Reagan et al. was proportionally the same in these patients as in the series as a whole. The results in these patients operated on by combined vaginal and abdominal hysterectomy had the same rate of survival but fewer complications than those given radical hysterectomy.

The favourable results from radiation therapy of carcinoma of the uterine cervix are well documented (4, 7, 10, 11, 16) but in some patients the primary tumour does not respond and eventually recurs. Treatment of persistent viable cancer recurrences has awakened much interest and different opinions have been presented as to how it could be carried out (1, 2, 3, 12, 15, 18).

This paper is an account of the results of hysterectomy in central treatment failures following radiation therapy of cancer of the uterine cervix in Stages I and II.

MATERIAL AND METHODS

The patient material consists of 977 cases of cancer of the uterine cervix in Stages I and II recorded during 1963-1972 (13). Radiation therapy was given using the Stockholm technique which was successively modified after 1969 to include high voltage treatment and fixed applicator-combinations enabling the placement of a well-defined known radiation dose in the tumour and in

the true pelvis (11). During the same period of time an increasing number of adenocarcinomas were treated surgically following preoperative radiation therapy.

Information on the tumour situation in the patients who have died due to their cancer is lacking in two cases.

Patients were considered to have central recurrence when after the conclusion of treatment tumorous growth was found in the uterus in the upper two-thirds of the vagina or in the proximal parts of the parametrium. Spreading to other organs and metastases on the pelvic walls were not considered when defining central recurrence. The patients have not been grouped according to progression of residual tumour or recurrence (5).

Central recurrence was recorded in 136 (14%) of the patients. It was clinically and technically possible to perform surgery in 50 (37%) cases.

The surgical procedure used during the first years was radical hysterectomy mainly using Okabayashi's method (14 patients). Eight of these patients were subjected to lymphadenectomy. During recent years a more moderate form of hysterectomy has been carried out separating the uterine vessels medially in the ureters without dissecting the latter. The operation is a combined vaginal and abdominal procedure (9) and permits the removal of a well defined optional part of the vagina. This technique was used in 13 patients. Lymphadenectomy was not performed.

Ordinary abdominal extrafascial hysterectomy was done in eleven cases. In four of these cases it was intended to perform radical hysterectomy but the presence of tumorous progression and/or pronounced postradiological fibrosis necessitated a more palliative therapeutic approach.

The tumour was so advanced in twelve patients that only exploratory laparotomy was performed.

Results of surgery expressed in persistent or recurrent growth of tumour in the area subjected to surgery, 2- and 5-year survival after operation for recurrence as well as fistulas between the urinary passages and the vagina and between the intestine and vagina have been recorded. The microscopy of both the biopsy specimens and of the operative specimens was re-evaluated in those cases where hysterectomy was performed and the cell morphology was classified according to Reagan et al. (15).

Table I Microscopical findings in surgical specimens

Type of operation	Vaginal resection not radical	Extensive tumour infiltration (>inner third of the cervix and/or tumour thrombosis)
Radical hysterectomy	1/14	9/14
Combined hysterectomy	1/13	10/13
Ordinary hysterectomy	5/11	5/11

RESULTS

Table I shows the results of the microscopical examination of the operative specimens in regard to the presence of tumour in the margins of the vaginal cuff, infiltration in the cervix and tumorous growth in lymph or blood vessels.

Colposcopy is now performed as clinical routine prior to combined hysterectomy but this was not done in case the vaginal resection was insufficient. At combined hysterectomy >3/4 of the vagina was removed in three cases and in one case 1/2 to 2/3 of the vagina. Colposcopy was not performed prior to the other hysterectomies.

Table II shows continued tumour progression and/or central recurrence in the area of surgery (central parts of the pelvis) and recurrence adjudged to be limited to the upper part of the vagina.

Table III shows the cell picture according to an et al among 76 patients who had hysterectomy and in 230 patients in Stages I and II during 0-1971 (8).

Attempts at curative treatment of recurrence following hysterectomy have only been made when the recurrence was limited to the upper part of the vagina. In three cases resection of the vagina was done and in two cases fulguration. These five patients are still alive. One of them has *in situ* changes in the vaginal introitus. The others are free from tumour.

Complications from the urinary passages and the rectum are seen in Table IV.

More than three fourths of the vagina was removed in the fistula patient who had combined hysterectomy.

Repair of the fistula and reimplantation of the ureters was performed in four of the fistula

patients and in one of them ureteroileostomy was performed using Bricker's method. One patient was operated on with ureterosigmoidostomy. A recto-vaginal fistula was repaired. These surgical measures were undertaken in the clinics of urology and surgery. The two patients last referred to had recurrences and died within one year. The others are still alive and free from tumour.

None of the patients succumbed during a period immediately following surgery (within 6 months). Table V shows 2 and 5-year survival after operation for recurrence.

DISCUSSION

The surgical methods used for treatment of central recurrence vary from exenterations of the true pelvis and radical hysterectomies to fulguration of the tumorous growth in the portio and in the vagina (1, 2, 3, 12, 16, 18).

Radical hysterectomy according to Okabayashi's method was employed during the early years. In introduction of high voltage therapy with relatively high absorbed radiation doses in the contents of the true pelvis caused pronounced fibrosis, however. This complicated or prevented the radical surgical approach which had been intended in some patients even when the spread of tumour was limited. The frequency of complications increased and postoperative retroperitoneal fibrosis with compression of the ureters occurred in some patients in connection with vesico-vaginal fistulas.

Conventional extrafascial hysterectomy gave unsatisfactory results except in those cases where central recurrence was diagnosed at a very early stage. The difficulties in removing a sufficiently large part of the vagina in fibrous tissues make this surgical technique most often unsuitable as a curative treatment method.

Table II Recurrence in the operative area

Type of operation	Central recurrence	Recurrence solely in the upper part of the vagina
Radical hysterectomy	5/14	-
Combined hysterectomy	5/13	3/13
Ordinary hysterectomy	5/11	4/11

Table III Distribution of patients according to

	36 patients with hysterectomy for recurrence 1963-1972	230 patients Stages I and II 1970-1971 (8)
Non-keratinizing ca.	22 (~61%)	170 (74%)
Keratinizing ca.	5 (~14%)	35 (15%)
Squamous cell ca.	1 (~3%)	6 (3%)
Adeno and adeno-squamous ca.	8 (~22%)	19 (8%)
Total	36	230

Also it can be difficult to decide abdominally where the vagina ought to be separated at radical hysterectomy. Combined vaginal and abdominal approach is a preferable alternative (9). We use laparoscopy for deciding where the vagina is to be divided and start the operation vaginally and there usually do an abdominal extrafascial hysterectomy (type II hysterectomy according to Rutledge) (16) or wide extrafascial hysterectomy according to Symmonds (18).

Adequate clearance of the vagina can be guaranteed in this way. The results have shown that this method gave adequate and free margins in all but one patient.

The frequency of pronounced infiltration in the cervical wall and/or tumour thrombosis was about the same for both the radical and the combined hysterectomies.

Recurrences were found in the area of surgery in the central part of the pelvis during the postoperative period at the same rate in patients subjected to either radical or combined hysterectomy. Recurrence in the upper part of the vagina alone was recorded in three out of thirteen cases after combined hysterectomy. It was not possible to ascertain recurrence in the upper part of the vagina after radical hysterectomy due to necroses which often remained during a relatively long period of time following surgery.

Recurrence in the vagina following hysterectomy can in many cases be treated successfully with local sections or fulguration.

One patient among those treated by radical hysterectomy and one of those who received conventional hysterectomy succumbed due to metastases in distant organs with no signs of tumour in the pelvis after four years and one year respectively.

The frequency of complications from the urinary tract and the intestine following radical hysterectomy was comparable to those found in the literature (16) but was lower in the patients treated by combined hysterectomy. This series of patients is however not large enough to permit any definite conclusions.

The cytological details of squamous cell cancer as characterized by Reagan et al (15) have been used as a guide for a more individualized treatment (6, 14, 17, 19). Johansson et al (8) were unable to show any obvious differences in tumour reaction to radiation therapy in a tumour material from 1969-1970. It was considered to be a representative sample out of the present tumour material from 1963-1972. The different cytological groups ought thus to be proportionally of the same size. The distribution of the three different types of squamous cell cancers was the same in the patients treated by hysterectomy for central recurrence as in the whole material from 1969-1970 (Table III). There are however proportionally more patients among those treated by hysterectomy who have an adeno-squamous cancer or adenocarcinoma. This agrees with the general opinion that adenocarcinoma has a poorer prognosis than squamous cell cancer.

Three patients with non-keratinizing carcinoma in the biopsy specimens had keratinizing carcinoma in the operative specimens and one patient with keratinizing carcinoma primarily had non-keratinizing carcinoma in the recurrent growth. In two patients with adeno-squamous carcinoma the recurrent tumour showed only adenocarcinoma. The microscopic morphology of the recurrent tumour thus coincides in most cases with that of the primary tumour.

The prognosis following operation for recurrence varies greatly in part due to the selection which is

Table IV Complications following operation for recurrence

Type of operation	Vesico-vaginal fistula	Recto-vaginal fistula	Total
Radical hysterectomy	4/14	1/14	5/14
Combined hysterectomy	1/13	0/13	1/13
Ordinary hysterectomy	1/11	0/11	1/11

Table V Two- and five year survival following operation for recurrence

Type of operation	2 year survival	5-year survival
Radical hysterectomy	9/14	5/13
Combined hysterectomy	9/12	5/8
Ordinary hysterectomy	4/11	3/10
Total	22/37 (~60%)	13/31 (~40%)

made and in part dependent on the way in which radiation therapy is administered Barber (1966) reported 40% 5 year survival after radical hysterectomy and 19.7% following exenteration Calame (1969) reported that three out of seventy patients given primary radiation therapy in Stages I and II were still alive after 5 years or longer following operation for recurrence Rutledge (1976) got 55% 5 year survival for central recurrence treated with radical hysterectomy and 28% 5 year controlrate for those operated on by exenteration Kottmeier (1971) reported 41.9% 5 year survival following radical hysterectomy and 42.7% when only fulguration was given The present material is limited but the 5 year survival is close to that of the two series mentioned above

Barber (1971) indicates a 17.4% 5 year survival for pelvic exenteration in cases with non involved nodes and only 5.1% survival with lymph nodes involved He stated that it was concluded from this study that survival is poor the complications so high and in the vast majority the survival so brief and difficult that pelvic exenteration is logically contraindicated in the group of patients with involved nodes complicating the recurrence

The extremely poor prognosis when nodes are involved would indicate that routine lymphadenectomy is not justified at operation for recurrence In central recurrence the tumour is often limited to the cervix and the upper part of the vagina when the diagnosis is made (16) Spread to the lymph nodes probably implies that the tumour has developed into a systemic disease and that it is no longer curable with surgery

CONCLUSION

Even though the present series of patients is small it seems to support the assumption that central recurrence after radiation therapy for invasive cervi-

cal cancer in Stages I and II can be treated locally with combined vaginal and abdominal extrafascial hysterectomy without lymphadenectomy The same rate of 5 year survival is achieved with fewer complications than have been reported for patients given radical hysterectomy

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WATER AND ION METABOLISM IN PLACENTA

1 *Water and Ion Content of Rabbit Placenta in Different Periods of Gestation*

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Abstract Total tissue water of rabbit placenta decreases 18% between the 9th day and the end of gestation. In same period intracellular water decreases by 40% and extracellular compartment swells. Total tissue sodium chloride content increase by 34% and 27% respectively from the 16th to the 33rd day while potassium decreases by 30%. The intracellular concentration of these does not vary greatly during gestation. Moreover the tissue magnesium content decreases by about 23% at end of gestation while its intracellular concentration is not show marked changes. Calcium content of rabbit placenta substantially increases during gestation and intracellular calcium shows a fivefold increase from the 16th to the 33rd day of gestation. The results are discussed in terms of relation between water and ion content and placental tissue growth and aging.

Our programme of investigation of physiological and metabolic characteristics of placenta (11-14) we have now undertaken a detailed study of water and ion metabolism of rabbit placenta at different degrees of maturity. The knowledge of the intimate mechanisms that regulate intracellular water and ion content would be of great interest in the comprehension of control systems which regulate the passage of many compounds across the placenta. Moreover a comprehensive set of information on this subject constitutes the basis for the study of pathological conditions of this organ such as placental insufficiency. In addition the morphological features of placenta (e.g. cytosyncytio-trophoblast) make these studies particularly interesting since they may reveal that basic physiological mechanisms such as regulation of cell volume and ion content and metabolism could be different from those acting in other cells.

We have been able to find only few often contradictory data on this problem in the literature (3, 5, 9).

In this paper the first part of the work dealing with the water and mineral ion content of rabbit placenta in different periods of gestation is reported.

Rabbit placenta was chosen since it easily provides a considerable amount of tissue at different ages of a reasonably short gestation. Moreover it is possible to get placentae even 2 or 3 min after killing the animal, a condition that is particularly useful since ion levels and equilibria are extremely sensitive to cell energy metabolism and oxygen supply to the tissue.

The results obtained show that in rabbit placenta from the 9th to the 33rd day of gestation (from the 31st to the 33rd day we deal of course with a post term gestation) total tissue water decreases by 18%, intracellular water also decreases by about 40% while the extracellular space swells. Total tissue sodium increases and total potassium decreases. K/Na ratio decreases gradually until it is reversed while the intracellular concentration of both ions does not vary significantly. The most impressive change is however related to calcium. Both total tissue and intracellular calcium show a marked increase during placental growth and senescence. Total magnesium content declines at the end of gestation whilst its intracellular concentration is kept constant.

The results will be discussed in terms of placental tissue aging.

Table I Ion content of rabbit placenta at different periods of gestation

Period of gestation (days)	Ion content (nmol/kg d wt)					
	Sodium	Chloride	Potassium	Magnesium	Calcium	Na/Cl ratio
I (9-15)	498 2±26 (45)	407 4±23 (45)	566 7±19 (44)	44 6±2 (42)	82 8±9 (39)	12 11
II (16-20)	375 2±16 (32)	310 2±7 (32)	521 8±11 (32)	44 3±2 (31)	61 5±6 (27)	12 14
III (21-25)	433 4±11 (20)	318 4±17 (17)	447 0±10 (20)	43 7±1 (15)	85 5±7 (16)	13 11
IV (26-30)	497 3±17 (15)	350 8±10 (14)	431 1±12 (15)	35 7±8 (14)	123 6±19 (14)	14 98
V (31-33)*	504 0±13 (24)	394 3±17 (23)	367 5±13 (23)	33 2±1 (24)	155 0±11 (9)	12 97

Mean±S.E. of the mean (no. of observations)

* Pregnancy has been prolonged beyond term (V period) by daily treatment with s.c. injection of 1000 IU chorionic gonadotrophin (HCG) from the 27th to the 32nd day of gestation

MATERIALS AND METHODS

Pregnant white giant New Zealand rabbits at known days of gestation were kept on standard diet. Some animals were brought beyond the physiological term of their gestation (30 days) by a daily s.c. injection of 1000 IU of human chorionic gonadotrophin (HCG) starting from the 27th day of their gestation. The study was carried out on placentae obtained from the 9th to the 33rd day of gestation. This interval has been arbitrarily subdivided into five periods as follows: period I from the 9th to the 15th day of gestation; period II from the 16th to the 20th; period III from the 21st to the 25th; period IV from the 26th to the 30th; period V from the 31st to the 33rd. Pregnancy was not further prolonged since about half of the fetuses were already found dead at the 33rd day. Furthermore, placentae of this last period showed extensive areas of necrosis and infarction.

Rabbits previously injected s.v. with 5000 IU heparin (1 ml Liqemin-Roche) were killed by cervical dislocation and bled by carotid incision. The blood was collected for ion determination. After bleeding, the abdomen was open and the pregnant uterus withdrawn. Placentae were carefully and quickly dissected from endometrium and amnion and care was taken to discard necrotic areas. Pieces were taken by cutting small pieces of tissue of about 100-150 mg which were gently blotted on hardened filter paper (Whatman no. 54) and placed in tared screw-cap plastic bottles. The bottles were weighed in order to determine the wet weight (w wt) and then dried overnight in an oven at 80°C. The day after, the bottles were weighed again after a period of equilibration at room temperature in a desiccator in order to determine the dry weight (d wt) and calculate the amount of tissue water expressed as kg/kg d wt.

For the analysis of ion content, the tissue was extracted by 0.1 N HNO₃ for at least four hours at room temperature, since this procedure has been shown to extract quantitatively all ions from many tissues (7). Ions were determined on the acid extract by atomic absorption spectrophotometry. For sodium and potassium, a final concentration of 0.06% of CsCl was added to standards and samples while for calcium and magnesium 1% LaCl₃ was added. Chloride content was determined spectrophotometrically at 460 nm following Zall et al. (24). Deionized water (Milli-Q Reagent Water System, Millipore Corporation, Bedford, Mass., USA) was used throughout the ex-

periments. aCl (fraction of tissue water chloride) at the same concentration of plasma calculated on the basis of plasma chloride concentration of 111.7±1.7 corrected by the Donnan factor for chloride of 0.977 (8, 15). Tissue water extracellular (H₂O)_e intracellular (H₂O)_i compartments were calculated by the expression $H_2O = H_2O_e \times aCl$ and $H_2O_i = H_2O(1 - aCl)$. Intracellular ion concentration was calculated by subtracting the amount of ion dissolved in the extracellular water at the same concentration as in the plasma from the total tissue ion content and dividing this value by the intracellular water.

RESULTS

Table I summarizes the ion composition of rabbit placenta at different ages of gestation. Sodium content decreases by 25% in period II of gestation; increases progressively by about 15% from period II to III and III to IV, in which the content stabilizes around 500 mmol/kg d wt through period V. These differences among the first four periods are statistically highly significant ($p < 0.0005$). The behavior of chloride strictly follows that of sodium and increases by 25% in period II and it increases to 310.2±7 to 394.3±17 mmol/kg d wt. Na/Cl ratio is kept constant at a value of 1.7-1.4. The concentration of potassium decreases gradually from 566.7±19 to 367.5±13 mmol/kg d wt (35%) at a probability level of 99.95% between each pair of periods. The ratio shows a progressive lowering from 1.4 (period II) to 0.7 (period V). The content of divalent cations markedly changes during placental growth. Calcium undergoes a substantial increase (35%) in period V and reaches a level of 155.0±11 mmol/kg d wt in period V. Magnesium decreases by 20% in periods IV and V.

The amount of total tissue water (H₂O)_t reported in Table II. It decreases from the first to

Tissue water Chloride apparent concentration in tissue water Fraction of tissue water contain at the same concentration of plasma water (α_{Cl}) and extracellular and intracellular water contents of rabbit placenta at different periods of gestation

H_2O_{wt} (kg/kg d wt)	$[Cl^-]$ tissue water ^a (mmoles/kg tissue H O)	α_{Cl}	H_2O (kg/kg d wt)	H_2O^d (kg/kg d wt)
6.2 ± 3 (44)	65.7 ± 6.8 ^f	575 ± 0.68 ^f	3.6	2.6
5.5 ± 0.6 (31)	56.4 ± 1.9	493 ± 0.74	2.7	2.8
5.2 ± 0.9 (17)	51.2 ± 4.3	535 ± 0.45	2.8	2.4
5.4 ± 0.7 (14)	50.0 ± 2.7	578 ± 0.63	3.1	2.3
5.1 ± 1 (74)	77.3 ± 4.9	676 ± 0.53	3.4	1.7

^a based on the basis of mean slices chloride content of Table I and mean total tissue water of this Table
^b α_{Cl} in tissue water/(concentration in plasma water) × (Donnan ratio) The Donnan factor for Chloride (0.977)
^c is the ratio of Cl^- distribution between plasma and tissue interstitial fluid (II) Plasma $Cl^- = 111.7 \pm 1.7$ (16)
^d kg plasma water

extracellular water = tissue $H_2O \times \alpha_{Cl}$
 intracellular water = tissue $H_2O \times (1 - \alpha_{Cl})$

^e S.E. (no. of observations)

^f calculated as error propagation from the original data Cl^- plasma total tissue H O Cl^- content of tissue

rod The difference is highly significant
 (0.5) On the basis of the above reported
 results we have calculated extracellular and
 intracellular water compartments. This determina-
 tion is usually done by the use of extracellular space
 markers such as inulin, polyglucose and others
 which markers require at least one to two hours
 of incubation in order to obtain a satisfactory equi-
 librium of the marker substance while all our tests
 were performed a few minutes after withdrawal
 of placenta. Therefore following other au-
 thors' water compartments were determined on
 the basis of tissue chloride content assuming that
 chloride is confined to the extracellular space.

The data obtained by following the latter assump-
 tion can give overestimated values since cellular
 membranes are not impermeable to chloride the
 predominantly extracellular distribution of this an-
 ion being determined mainly by a membrane poten-
 tial negative at the interior (2, 6). Once membrane
 potentials directly measured by electrophysiologi-
 cal methods are known it is easy to derive an
 expression to correct for intracellular chloride (15).
 We were not able to bring such correction to our
 calculation since we have not found any published
 values of placental cell membrane potential α_{Cl}
 determined in placentae at different periods of
 gestation are reported in Table II together with

Table I. Calculated intracellular concentrations of sodium, potassium, calcium, magnesium and calcium for sodium and potassium of rabbit placenta at different periods of gestation

Intracellular concentration (mmoles/kg intracellular water)					E_m^a (mV)	E_m^b (mV)
	Sodium	Potassium	Calcium	Magnesium		
5)	4.2	207.5	23.6	15.6	-	-90.4
0)	4.5	181.9	16.4	15.0	-	-94.0
5)	14.7	178.7	28.7	17.0	6.7	-94.5
0)	25.2	177.7	45.4	14.0	47.9	-94.7
3)	6.8	211.1	81.8	18.1	84.0	-89.9
	14.7 ± 9.1 ± 9 (16)	5.5 ± 0.3 (16)	5.8 ± 0.2 (78)	11.96 ± 0.03 (75)		

^a Cellular concentrations have been calculated on the basis of mean water and ion content of Tables I and III and
^b on concentrations of this Table

membrane potential for Na and K = $\frac{RT}{F} \times \log \frac{[Na]_i}{[Na]_o}$ or $\log \frac{[K]_i}{[K]_o}$
^c g/kg plasma water (Plasma $H_2O = 93.1 \pm 1.4$ g/kg plasma)

Cl^- in total tissue water and with the calculated size of both extracellular and intracellular compartments. From periods II to V αCl gradually increases up to a maximum of 0.676 ± 0.053 . The amount of extracellular water accordingly increases from 2.7 to 3.4 kg/kg d wt (+26%) despite the lowering of total tissue water. On the contrary the intracellular water decreases gradually from periods II to V by 40%.

Data of Table III were calculated on the basis of the above reported water and ion values. The intracellular concentration of each ion was calculated from the size of intracellular compartment (Table II) and plasma ion concentration (last row of Table III). Furthermore in the two last lines membrane potentials for Na^+ and K^+ calculated from the Nernst equation are also reported. Intracellular concentrations of potassium and magnesium do not vary greatly during the course of pregnancy while $[\text{Na}^+]_i$ increases from the values that approach 0 in the first two periods to the higher levels found subsequently. However the most striking changes concern $[\text{Ca}^{++}]_i$. The apparent concentration of this cation increases progressively from 16.4 (period II) to 81.8 mM (period V). The calculated membrane potential for K^+ (E_{mK^+}) indicates that no substantial variation of this parameter takes place during placental growth and senescence. Cell membrane potentials for Na^+ show the expected changes due to the different intracellular sodium found in the different periods.

DISCUSSION

Tissue water

The results reported show that physiological growth of rabbit placenta is characterized by a decrease and redistribution of total tissue water. The older the tissue becomes the more its water content decreases down to a drastic decrease in the senescent placenta of the past term pregnancy. An interesting feature is that the loss of water is accompanied by an expansion of the extracellular compartment as documented by the increase of αCl that starts from the period II. This can be explained by the more abundant collagen synthesis that takes place during growth in the villous axis. Furthermore the decrease of total tissue water appears to be the sum of a substantial decrease of the intracellular compartment (about 40%) and a smaller increase (25%) of the extracellular fraction.

Our results can be interpreted as an example of placental involution and senescence according to the well known relation between tissue size and aging.

Ion content

In this work we took into consideration the properties of mammalian cells. The major sodium and potassium gradients is in fact, an energy dependent property of these cells. Moreover calcium and magnesium intracellular concentrations are always more evidently related to regulation of cellular metabolism.

From data of Table I it appears that placental αCl is strictly correlated to the total tissue ion content although with some differences among the ions. Sodium increases significantly and progressively from period II to the end of pregnancy whereas potassium decreases. The possibility of these changes can be due to tissue blood flow more abundant toward the end of gestation is not out if one considers that they are consequences of an increase of red blood cells which are more numerous in older placenta. On the contrary we could estimate that the increase of erythrocytes induces an overestimation of the changes observed (placental αCl could be much lower in older placenta). Intracellular concentration of potassium does not vary greatly as shown also by calculated E_{mK^+} values. In default of directly measured intracellular values are the only available for these cells but fall perfectly in the range of potentials measured in other cells (e.g. muscle cells). Intracellular concentration of sodium behaves in a different way. In period II its concentration is about zero and it becomes higher in period V. Sodium is usually outside the cell by an active pump (the Na⁺/K⁺ stimulated ATPase). This is also probably the case in placental cells which extrude sodium against a gradient sustained by the plasma concentration of about 140 mM. The increase of intracellular sodium concentration may indicate that the efficiency of the extruding pump is failing and this would be consistent with a documented decrease of the energy production system in ageing placenta. Intracellular tissue magnesium has a break point between periods II and III and the following periods but as far as its intracellular concentration is concerned it remains unchanged at a level of about 16 mM. The consequent imbalance of the intracellular magnesium

versus plasma concentration (0.96 mM) as in other mammalian cell systems (8) is possibly concentrated in the cell interior may be prevalently bound to subcellular structures (18). Calcium behaves differently in placenta as compared to other tissues. This cation is a major extracellular constituent (8) being regulated by energy dependent mechanisms (10) or hetero-exchange diffusion processes (11). Our determinations the level of both total and intracellular Ca^{2+} are much higher than plasma values. It is widely acknowledged that calcium is easily bound to necrotic tissue areas. In the case of the placenta this can be true in the two periods studied but is extremely unlikely to be the case in the preceding periods in which placenta is completely free of necrosis and infarction. However the possibility of microfoci of necrosis still remains. With regard to the high content of this organ the peculiar role of the placenta plays in the transfer of calcium from maternal serum to fetus must be taken into account. Calcium is actively transported by placental cells (4, 23) and this hypothesis is supported by the evidence of a Ca^{2+} -dependent ATPase in plasma membranes of this organ (20, 21).

Our results in the placental ages grouped in the first half require an additional discussion. In this study in fact some of the parameters we have analysed (i.e. total tissue sodium and chloride, total water and intra-extracellular water compartment) do not fit with the sequentiality that comes from the data analysis of the following groups. In the first of all to underline the technical difficulties related to the study of placentae at the intermediate 9 and 15 days. The exiguity of placenta renders unavoidable contamination by surrounding tissues such as endometrium and membranes. Moreover we could speculate that the observed differences are linked to the peculiar physiological conditions of placenta in the first half of pregnancy when cell growth prevails over tissue involution and senescence.

In the results of the study we have undertaken on the water content and ionic composition of rabbit placenta in different periods of gestation lead to the following conclusions: placental growth and involution are characterized by a decrease of total water content accompanied by both the decrease of the intracellular water compartment

and the swelling of the extracellular compartment. As far as the monovalent cation content (Na^+ and K^+) is concerned we can say that only sodium shows a clear increase throughout the placental growth while potassium content reflects the changes in size of extracellular and intracellular water compartments. Magnesium undergoes a clear decrease toward the end of pregnancy while calcium shows a fivefold increase from the 16th to the 33rd day of gestation.

Further studies are in progress with the aim of understanding the observed changes from a functional and metabolic point of view.

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SHORT COMMUNICATIONS

EARLY PRENATAL CHROMOSOME ANALYSIS
OF 604 FETUSES

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Chromosome analysis may now be considered routine which should be offered to women at risk of having children with chromosomal diseases. Few series are available. Large series are necessary for the evaluation, for example, of the cost-benefit of the procedure. Therefore the results of fetal chromosome analysis in 604 unselected pregnancies are presented. The value for the individual pregnancy is already obvious. Further details will be published later.

Chromosome analysis was carried out when amniocentesis was done for other purposes in the second trimester, e.g. for alpha-feto-protein-determination in women at risk of having neural tube defects. In a number of cases women came to the hospital with the expressed wish of having a fetal chromosome investigation done for a number of other reasons. The test was carried out whenever possible. Only very few of those offered the analysis refused to have it, and there was no reason to believe that these had a different risk from those having the investigation.

Amniocentesis was carried out around the 14th-15th week, sometimes later. Ultrasound was used in all cases (7). Amniotic cell cultures were carried according to methods earlier published (15). Results were obtained in all cases.

In three cases twins were diagnosed by ultrasound and amniotic fluid obtained from both fetuses. In three cases twins were present but not diagnosed.

MATERIAL AND METHODS

In March 1973 to February 1976 chromosome analysis was offered to all pregnant women referred to our hospital at risk of having a chromosomally abnormal child. Fur-

Table 1. Elevated risk of chromosome abnormality

	No investigated	No abnormalities	
Maternal translocations	3	0 (3)	-
Maternal disease	15	(8 males)	53
Partner has chromosome abnormality	3	0	-
Partner 40 years or more	133	5 1 47,XY +13 1 47,XX +18 2 47,XX +,-1 1 47,XY +21 (1 46,XX ?p+ 6p-)	37
Partner 35-39 years	208	1 1 47,XX +21 (1 46,XX t (17/22))	0.4
Partner earlier born child with chromosome abnormality	37	0	-
	399	6 (14)	1.5 (3.5)

Table II *X linked diseases (8 male fetuses 5 abortions)*

No abortion in 3 cases

- 1 Haemophilia diagnosis not confirmed when patient admitted for abortion from other hospital
- 2 Patient had 2 male children with congenital malformations of sex organs Did not want abortion Had malformed male child
- 3 Father had haemophilia Parents wanted only boys

Table III *Normal risk of chromosome abnormality*

	No investigated	No abnormalities	%
Previous child with congenital malformations inborn errors of metabolism oligophrenia or other diseases	37	0	-
Previous child with spina bifida anencephaly hydrocephaly	44	1 (47,XYX)	2.2
Mother has spina bifida hydrocephaly	2	0	-
Down's syndrome in the family	49	1 (47,XXX)	2.0
Patients with investigation	34	0 (45,XX) (14/22)	-
Other	39	0	-
	205	2	0.9

A total of 604 fetuses from 601 pregnant women were examined. The patients were divided in two groups: group one having a higher than normal and group two a not higher than normal risk of having a child with a chromosome abnormality.

Table V

	Literature survey			German survey			European survey	
	No invest	No abnorm	%	No invest	No abnorm	%	No invest	No abnorm
Familial translocations	91	13	14	36	4	11	179	17
X linked diseases	149	75	50	16	7	43	280	174
High maternal age	1 404	41	2.8	728	22	3.6	1 293 (2 269) ^a	63
Earlier chromosome abnormality	630	11	1.7	295	11	3.7	1 047	14
Other indications	776	12	1.5	193	(1)	(0.5)		

^a 35 or 40^b Age information only available for 1 293Table IV *References cited*

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 Milunski et al 1974
 Nier Meijer 1975
 Philip et al 1974
 Laurence & Gregory 1976

Group 1 Elevated risk of chromosome abnormality

Table I shows that 6 chromosome abnormalities (balanced translocations) were found among 39 cases (1.5%). In 8 of 15 cases with a risk of X linked disease a male fetus was ascertained. 14 (3.5%) had thus an indication for abortion but only 11 abortions were carried out (Table II).

Group 2 Normal risk of chromosome abnormality

Table III shows that two chromosome abnormalities (one balanced translocation (not familial) was found) in 11 abortions (0.9%) were carried out.

DISCUSSION

The numbers in each of the groups are small. This is also true for those results available in the literature (Table IV). By evaluation of the results from the literature and those from an unpublished large series from Germany and the collected results from

on of risks based on available

tris	4-14%
dis	43-53%
age 1 ears	17-37%
urrence risk	(0-) 17-27%

European Centers (Galjard in press) (Table V) : figures for risks are obtained (Table VI) figures may be representative and may be the analysis of cost effectiveness. This is to be of importance at the present time in Western countries where research funds for chromosome analysis are no longer available.

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LINKED VALUES OF E ROSETTE FORMING LYMPHOCYTES IN MOTHER AND NEWBORN

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The T-cell marker of spontaneous rosettes with lymphocytes was determined in 34 normal term-borns on the peripheral lymphocytes of the mother corresponding cord blood lymphocytes of the immediately after delivery. A consistently lower E-rosette forming lymphocytes was observed in compared to female newborns in different parity and in twin deliveries. When the mean values were determined for corresponding E-rosettes of maternal and newborn lymphocytes in each group (female and male of offspring) they were nearly identical.

cell marker of spontaneous rosettes with erythrocytes has been studied by various investigators in the cord blood of healthy infants and values ranging from 35-53% are reported (2). In a previous study we reported some observations concerning the possibility of a balanced relationship between maternal and newborn E-rosette forming lymphocytes (4). In groups of maternal newborn pairs were found in one group III percent of E-rosette forming lymphocytes was high in the mother and low in newborn (58% and 16%) while the opposite relationship was obtained in the second group (43% and 37%). At the same time the mean values of rosette forming cells in each group was similar (47% and 41% respectively). The aim of the present investigation was to find if this fetomaternal relationship could be correlated with parity or with the sex of the newborn.

PATIENTS AND METHODS

Following essentially the method of Benirsch et al. (1) spontaneous rosettes with sheep erythrocytes were determined on the peripheral lymphocytes of the mother and the corresponding cord blood lymphocytes of the newborn

immediately after delivery in 34 normal term-deliveries. Of the 34 mothers studied 11 were after their first delivery, 10 after the second, 9 after third or subsequent and 4 after twin deliveries (dizygotic twins, 3 cases with male-female offsprings and one case with two male offsprings).

E-rosettes in twins were determined separately from each placenta. For the calculation of the mean percentage of E-rosette forming T-cells 300 lymphocytes were counted for each mother and corresponding newborn.

RESULTS

A consistently lower level of E-rosette forming lymphocytes was observed in male newborns (34-45% mean 37%) as compared to female newborns (42-55% mean 44%) in all parity groups including twin deliveries.

In contrast to all other groups the mean percentage of E-rosette forming cells in twin deliveries (male and female offspring) was higher in the newborn than in the mother. In all 4 cases of twin deliveries the mean value of E-rosette forming cells of the mother was below 50% and lower than for the other groups (see Table I).

The mean values of maternal and newborn E-rosette forming lymphocytes for each group (females and males) were nearly identical (47% and 44% respectively). Similar mean values were obtained in our previous experiment (4).

DISCUSSION

The existence of a consistently lower percentage of E-rosette forming lymphocytes in male compared to female newborns suggests a possible role for sex-linked histocompatibility antigens in the fetomaternal relationship (5, 6). A decreased T

11 keto etiocholanolone	<0.91 μmol
11 hydroxy androsterone	2.2 μmol
11 hydroxy-etiocholanolone	0.51 μmol
A/E ratio	0.86
11 ratio	8.0

SURGICAL OBSERVATIONS

13.5 l of yellow brown opaque fluid were withdrawn by abdominal puncture. The fluid was aseptically free from cellular elements and composed of protein colloidal particles. Laparotomy revealed a large flabby thick walled cyst attached by a suspensory ligament to the right side. On the left side a 4x2.5x2 cm whitish formation also attached by a suspensory ligament was found. Neither uterus nor uterine tubes were present. Small grain seed like objects were found in the pouch of Douglas and up along both sides, samples of which were taken for biopsy. The removed cyst weighed 2950 g and contained a pale fluid. An object which was firm and the size of a small hen's egg was found embedded in the cyst wall.

The following patho-anatomical description was given (Dr Love Jepsen). The cyst's wall was composed of a thick layer of collagen connective tissue. The luminal side was lined by a single layer of epithelium composed of low cylindrical to cubical cells, the nuclei of which were round and regular. Small papilliform structures as well as small psamoma like bodies were scattered in the luminal layer. Below the epithelium a thin layer of macrophages was found. Histological diagnosis: Mesothelial cyst.

Peritoneal biopsy showed adipose and connective tissue enclosed cysts. The lining of these cysts was a mesothelial like epithelium within which were scattered small psamoma like structures. Neither epithelioid granuloma nor malignant changes were found. Histological diagnosis: Mesothelial cyst. Both gonads were testicular structures corresponding to testicular feminizing of complete type.

DISCUSSION

Heidenreich in 1975 (2) described a patient originating from the right gonad lay embedded in the wall of a child's head sized cyst. The cyst was composed of collagen connective tissue with an outer coat of smooth muscle cells and a lining lined with a single layer of cubical and cylindrical cells. The patient's vagina was about one finger length. Heidenreich proposed that the cyst had developed from the Mullerian tubular system and suggested that it resulted from failure of testis inhibition (Oviduct repressor - Mullerian inhibiting substance) of the development of the Mullerian system.

Several similarities between the case reported in this paper and that described by Heidenreich were seen but since no rudimentary remnants of the Mullerian tubular system were found we are unable to support the hypothesis proposed by Heidenreich.

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Submitted for publication Dec 7 1976

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WHO RECOMMENDED DEFINITIONS TERMINOLOGY AND FORMAT FOR STATISTICAL TABLES RELATED TO THE PERINATAL PERIOD AND USE OF A NEW CERTIFICATE FOR CAUSE OF PERINATAL DEATHS

Modifications Recommended by FIGO as Amended October 14 1976

The following limits for the inclusion of births and perinatal death in statistics are recommended

That all fetuses and infants delivered weighing 1000 g or more be reported in the country's statistics whether or not they are alive or dead. It is recognized that legal requirements in many countries set different criteria for registration purposes. It is hoped that the countries will arrange the registration or reporting procedures in such a way that the events required for inclusion in the statistics can be identified easily (WHO—Approved by FIGO)

That mortality statistics reported for purposes of international comparison should include only those born weighing 1000 g or more (WHO—Approved by FIGO)

Perinatal Statistics

Birth Weight

The first weight of the fetus or newborn obtained at birth. This weight should be measured preferably within one hour of life before significant postnatal weight loss has occurred (WHO—Approved by FIGO)

Low Birthweight

Less than 1500 g (WHO—Modified by FIGO by deleting the words 'up to and including 2499 g')

Gestational Age

The duration of gestation as measured from the first day of the last normal menstrual period. Gestational age is expressed in completed days or completed

weeks (e.g. events occurring 280 to less than 287 days after the onset of the last normal menstrual period are considered to have occurred in 40 weeks of gestation)

Measurements of fetal growth as they represent continuous variables are expressed in relation to a specific week of gestation (e.g. the mean birth weight for 40 weeks is the one obtained at 280 days—less than 287 days of gestation on a weight for gestational age curve) (WHO—Accepted by FIGO)

2.4 Perinatal Period

The perinatal period is the one extending from the gestational age at which the fetus attains the weight of 1000 g (equivalent to 28 completed weeks of gestation) to the end of the seventh completed day (168 completed hours) of life (WHO—Approved by FIGO is the word 'completed' included where underscored)

2.5 Pre Term

Less than 37 completed weeks (less than 259 completed days) (WHO—Accepted by FIGO)

2.6 Term

From 37 completed weeks to less than 42 completed weeks (259 to 293 days) (WHO—Accepted by FIGO)

2.7 Post Term

Forty two completed weeks or more (WHO—Approved by FIGO)

2.8 Birth

Complete expulsion or extraction from its mother of a fetus irrespective of whether or not the umbilical

11 keto etiocholanolone	<0.91 μ mol
11 hydroxy androsterone	2.2 μ mol
11 hydroxy-etiocholanolone	0.51 μ mol
A/E ratio	0.86
11 ratio	8.0

SURGICAL OBSERVATIONS

13.5 l of yellow brown opaque fluid were withdrawn by abdominal puncture. The fluid was aseptically free from cellular elements and composed of protein colloidal particles. Laparotomy revealed a large flabby thick walled cyst attached by a suspensory ligament to the right side. On the left side a 4×2.5×2 cm whitish formation also attached by a suspensory ligament was found. Neither uterus nor uterine tubes were present. Small grain seed like objects were found in the pouch of Douglas and up along both sides, samples of which were taken for biopsy. The removed cyst weighed 2950 g and contained a pale fluid. An object which was firm and the size of a small hen's egg was found embedded in the cyst wall.

The following patho-anatomical description was given (Dr Løve Jepsen). The cyst's wall was composed of a thick layer of collagen connective tissue. The luminal side was lined by a single layer of epithelium composed of low cylindrical to cubical cells, the nuclei of which were round and regular. Small papilliform structures as well as small psamoma like bodies were scattered in the luminal layer. Below the epithelium a thin layer of macrophages was found. Histological diagnosis: Mesothelial cyst.

■ peritoneal biopsy showed adipose and connective tissue enclosed cysts. The lining of these cysts was ■ mesothelial like epithelium within which were scattered small psamoma like structures. Neither epithelioid granuloma nor malignant changes were found. Histological diagnosis: Mesothelial cyst. Both gonads were testicular structures corresponding to testicular feminizing of complete type.

DISCUSSION

Heidenreich in 1975 (2) described a patient whose right gonad lay embedded in the wall of a child's head sized cyst. The cyst was composed of collagen connective tissue and an outer coat of smooth muscle cells and a lining lined with a single layer of cubical and cylindrical cells. The patient's vagina was about one finger length. Heidenreich proposed that the cyst had developed from the Mullerian tubular system, and suggested that it resulted from failure of testicular inhibition (Oviduct repressor Mullerian inhibiting substance) of the development of the Mullerian system.

Several similarities between the case reported in this paper and that described by Heidenreich can be seen but since no rudimentary remnants of the Mullerian tubular system were found we are unable to support the hypothesis proposed by Heidenreich.

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Neonatal Mortality Rate

Neonatal deaths of infants
 1 1000 g and over at birth

Infants weighing 1000 g

er

—Approved by FIGO)

Stillbirth Rate

Infants weighing 1000 g

er

Infants weighing 1000 g

er+live born infants weighing

and over at birth

—Approved by FIGO with the suggested

cation of adding the italicized words at

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have been completed the 2nd week when 14 days have been completed and so on FIGO suggests the following technique for designating weekly intervals

Gestational Age

28 weeks to less than 32 completed weeks (196 days to less than 224 completed days)

32 weeks to less than 36 completed weeks (224 days to less than 252 completed days)

36 weeks to less than 38 completed weeks (252 days to less than 266 completed days)

38 weeks to less than 42 completed weeks (266 days to less than 294 completed days)

42 completed weeks (294 days) and over

5.3 For early neonatal deaths by age of death using the following intervals

Birth to less than 60 completed minutes

1 hour to less than 12 completed hours

12 hours to less than 24 completed hours

24 hours to less than 48 completed hours

48 hours to less than 72 completed hours

72 hours to less than 168 completed hours

Where detailed information is not available data on age at death should be provided as follows

Birth to less than 60 completed minutes

1—less than 24 completed hours

24—less than 168 completed hours

(WHO—Modified by FIGO)

In each table appropriate totals and subtotals should be given (for example all infants with birth weight 1000 up to less than 1500 g or all infants of 28 to less than 38 completed weeks gestation etc (together with appropriate percentages. If more detailed breakdowns are tabulated it should be possible to aggregate them into the above groupings (WHO—Approved by FIGO with the italicized suggested modifications)

6.0 Mortality Statistics

These should be presented in relation to different groups of infants using the definitions of rates given below

6.1 Stillbirth and Perinatal Death Rates

(a) all infants 1000 g or more

(b) all infants 1000 g or more in 500 g groups

11.3 While the supplementary information to be collected at death or stillbirth may be varied in accordance with the wishes of the individual countries it is recommended that consideration be given to the collection of the following items as a minimum

Mother

Date of birth

Previous history

Number of previous pregnancies live births/
stillbirths/abortions

Outcome of previous pregnancies live births/
stillbirths/abortions and date
(WHO—Approved by FIGO)

GYNECOLOGIC TERMS AND DEFINITIONS

2.1 *Postmenstrual phase*

(*Menstrual period Menstruation Menses*)

The postmenstrual phase is the phase that includes the 4–5 days following the menstrual phase. The endometrium is thin measuring ordinarily only 1 or 2 mm in thickness. The surface epithelium and the epithelium lining the glands is of cuboidal type. The endometrial glands are straight narrow and collapsed the stroma is dense and compact.

2.2 *Proliferative Phase*

The proliferative phase is the growth phase of the endometrium. The endometrium is stimulated by estrogen. The endometrial glands are straight and short and the glandular epithelium is cuboidal and shows no evidence of secretory activity. The basal cells multiply and the spiral arteries begin to grow.

2.3 *Secretory Phase*

The secretory phase is the postovulatory phase of the endometrium. The endometrium is stimulated by estrogen and progesterone. The endometrial glands are long and tortuous. The glandular epithelium is columnar and filled with secretion. The stromal cells are large and the spiral arteries are long and tortuous.

2.4 *Premenstrual Phase*

The premenstrual phase is the phase that includes the 2–3 days prior to the menstrual phase and corresponds to the regression of the corpus luteum.

The chief histologic changes are: infiltration of the stroma by mononuclear leukocytes producing inflammatory appearance (inflammatory framework of the stroma) and disintegration of the stroma. As a result of the disintegration and secretion the thickness of the endometrium often decreases significantly during the premenstrual phase. In the premenstrual phase the glands and arteries collapse.

2.5 *Menstrual Phase—Menstrual Period*

The menstrual phase is the period of shedding of the endometrium. There are many types of polymorphonuclear leukocytes, plasma cells and other wandering blood cells in the tissue.

2.6 *Amenorrhea*

Amenorrhea is the absence of menstruation. It may be primary or secondary, physiologic or pathologic. It is a subjective but not a reliable sign of pregnancy.

2.7 *Amenorrhea Pathologic*

Pathologic amenorrhea is the cessation of menstruation for at least 3 months at any time after puberty other than during pregnancy and lactation. It may occur before the onset of menopause. It can be either primary or secondary and caused by any of the following factors: congenital abnormalities, central nervous system lesions, systemic conditions, endocrine disturbances and uterine trauma.

2.8 *Amenorrhea Physiologic*

Physiologic amenorrhea is the normal absence of menstruation before the menarche, during pregnancy and lactation and after the menopause.

2.9 *Anovular Menstruation (Anovular Bleeding)*

Anovular menstruation is menstrual bleeding without discharge of an ovum.

2.10 *Dysmenorrhea*

Dysmenorrhea is a symptom characterized by painful menstruation.

2.11 *Mechanical Dysmenorrhea*

Mechanical dysmenorrhea is a menstrual pain due to cervical stenosis or other obstruction to menstrual flow.

unctional Dysmenorrhea

hea : menstrual pain observed
y nc : worthy pelvic lesion

Acquired Dysmenorrhea

rrhea is menstrual pain caused
on : lvic disease

typ : norrhea

enorhea is a diminution in the amount of
or a shortening of the duration of menstrua

ittelschmerz

chmerz is intermenstrual pain in the lower
in generally associated with ovulation

ligomenorrhea

enorhea is a reduction in the frequency of
ation. An interval between the cycles of
than 38 days but less than 3 months indi
ligomenorrhea

uberty

r is the period when a person becomes sexu
ture. The reproductive organs become func
and secondary sex characteristics are de
l

icarious Menstruation

us menstruation is bleeding from any
other than the mucous membrane of the
cavity. It occurs periodically at the time
ormal menstruation should take place

ibruptio Placentae

io placentae is the complete or partial de
nt of the normally implanted placenta from
ine wall in 20 weeks or more of gestation
io placentae may occur in conjunction with
a previa. Hypofibrinogenemia is the most
in complication of abruptio placentae

2 20 Concealed Hemorrhage

A concealed hemorrhage is an accumulation of
blood within the uterus or amniotic sac associated
with abruptio placentae

2 21 Placenta Previa

Placenta previa is the implantation of any part of the
placenta in the lower part of the uterine segment.
The term expresses the anatomic relationship be
tween the placental site and the lower uterine seg
ment. The placenta encroaches on or covers (com
pletely or partially) the internal cervical os.
Placenta previa is classified as marginal, partial or
total.

2 22 Marginal Placenta Previa

Marginal placenta previa is present when some part
of the placenta is attached to the lower uterine
segment and extends to but does not cover any
part of the internal cervical os.

2 23 Partial Placenta Previa

Partial placenta previa is present when any part of
the placenta incompletely covers the internal cervi
cal os.

2 24 Total Placenta Previa

Total placenta previa is present when any part of
the placenta completely covers the internal cervi
cal os.

2 25 Abortion

Abortion is the expulsion or extraction from its
mother of a fetus or embryo weighing 500 g or less
(approximately equal to 20 completed weeks (140
completed days) to 22 completed weeks (154 com
pleted days)) of gestation or an otherwise product of
gestation of any weight and specifically designated
(e.g. hydatidiform mole) irrespective of gestational
age and whether or not there is evidence of life and
whether or not the abortion was spontaneous or
induced.

ANNOUNCEMENTS

An International Symposium on Genito-Urinary Trichomoniasis will be held in Paris 8-9 July 1977

For all information please address to Doctor A Fari S I T G U 5 Blvd de Strasbourg 75010 Paris France

The Fourth UICC Training Course in Cancer Research arranged by the International Union Against Cancer will take place on 4-17 September 1977 in Budapest

For further information write Dr László Holczinger Research Institute of Oncopathology 1122 Budapest Ráth Gy str 7 Hungary

The XVI Congress of the Spanish Fertility Society and International Symposium on Cervical Factors organized by the Spanish Fertility Society in Barcelona will take place on the 1st 2nd 3rd 4th and 5th November 1977 Please note that the dates have been changed Official reports Endoscopy on fertility Andrologic diagnoses and natural methods of family planning Round Table and courses for postgraduates

For further details apply to: Secretary General Dr Louis C Pous Ivern Calle Muntaner 292-294 1a Barcelona 6 Spain

Conference on the biology of day international workshop con Montreal in May 1978 An ference will be to acquaint clinicians w regarding our understanding of connective ground substance while allowing ' greater insight into clinically important the incompetent cervix induction of labor and dilatation for induced abortion To achieve plinary approach we solicit in the biochemistry physiology and anatomy of the dilative tissue as these relate to the dilatation and from workers in who are measuring the forces of dilatation new types of dilators study vical dilatation or related areas of investigation expenses will be paid for invited participants I welcome in any time Abstracts should be December 1 1977 to Phillip G Stubblefield ton Hospital for Women 221 Longwood Avenue Boston Mass 02115 USA or Frederick Naftolin MD Women's Pavilion Royal Victoria Hospital 68 Avenue West Montreal PQ Canada H3A 1A1

Letter to the Editor

of pain in primary dysmenorrhoea by
adrenoceptor stimulating drugs

recent report in this journal on the use of isoxsuprine in essential dysmenorrhoea (6) the authors concluded that β adrenoceptor stimulation is effective in relieving menstrual pain. This conclusion was based on the results of a double blind controlled clinical trial of isoxsuprine. The patient material of the investigation was limited, consisting of 26 women of whom 26 completed the trial. No information on the gynaecological status of the patients was given and only one dose regimen of isoxsuprine ($10 \text{ mg} \times 3$) was tried.

For obvious reasons the results obtained in this study cannot be accepted as evidence for lack of relieving effect of β adrenoceptor stimulators in primary dysmenorrhoea. In previous studies of women suffering from this condition it was found that the myometrial activity was increased compared with that found in normal women (1, 2, 3, 7). It has also been demonstrated (2) that the uterine blood flow decreased in association with the increased myometrial activity and that the pain was probably caused by uterine ischemia (see also Fig. 1).

It is well known that the myometrium contains receptors of β_2 -type and that stimulation of these receptors results in relaxation of the uterus. β adrenoceptor stimulators have been increasingly used clinically for depressing uterine activity in the pregnant uterus (for ref. see (4)). In both non-pregnant women (1) and in women with primary dysmenorrhoea (2) the selective β_2 adrenoceptor stimulator terbutaline was found to decrease the uterine activity and increase the uterine blood flow. When this occurred in the women with primary dysmenorrhoea they reported complete relief of pain (Fig. 1).

Thus there is evidence that β_2 adrenoceptor stimulation can relieve menstrual pain effectively. However a prerequisite for this effect is that the

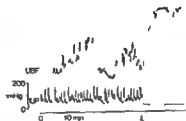


Fig. 1 Uterine blood flow (UBF), intrauterine pressure (IUP) and the effect of terbutaline (0.5 mg i.v. injection indicated by arrow) on the first menstrual day in a woman with severe primary dysmenorrhoea. One minute after the injection the patient reported total pain relief. (From Åkerlund et al. Br J Obstet Gynaecol 83: 673, 1976.)

β -adrenoceptor stimulator used reaches the uterus in sufficient amounts. There was no proof of this in the study cited above (6). In our opinion, therefore, the conclusion that can be drawn is not that β adrenoceptor stimulation is ineffective for relieving menstrual pain, but that isoxsuprine in the dosage used probably lacks a therapeutic effect in this condition.

Unfortunately because of their side effects, primarily tremor, the β adrenoceptor stimulators presently available seem to be unsuitable for treatment of primary dysmenorrhoea (2, 5).

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FESTSCHRIFT

to

PROFESSOR

Axel Ingelman-Sundberg

in Recognition of

his Professorship in

Obstetrics and Gynecology

at Karolinska Institutet

and

his Chief Editorship of

Acta Obstetrica et Gynecologica

Scandinavica



AXEL INGELMAN-SUNDBERG

When Axel Ingelman Sundberg on July 1st 1977 leaves the post of Chairman of the Department of Obstetrics and Gynecology at the Sabbatsberg Hospital he will be able to look back with great satisfaction upon a long period of fruitful work including research education and medical service

Ingelman Sundberg obtained a sound obstetrical and gynecological training under Professor Axel Westman who had a unique ability to stimulate his disciples to scientific work. To work in this research environment was highly encouraging and turned out to be an excellent foundation for future chairmen of women's clinics at universities and general hospitals

From a scientific point of view Ingelman Sundberg has treated a number of problems within the sphere of obstetrics and gynecology. Thanks to an unusual technical and surgical capability combined with a creative imagination he has enriched the speciality with several important methods of treatment particularly in the urological area. He has deepened our knowledge in this field via anatomical and roentgenological observations and has made a great contribution with his investigations into the cause and treatment of urinary incontinence urethrovaginal vesico-vaginal and recto-vaginal fistula. From these findings he has worked out ingenious surgical methods for the healing of incontinence conditions whether they be caused by sphincter damage pelvic floor insufficiency fistulas or nerve damage

He has moreover been a successful scientist in other fields of research and together with experts in pharmacology endocrinology and medical biophysics made interesting discoveries. In the long row of publications prominence should be given to a penetrating analysis of the role of vitamin E among other things for sexual functions placental development and sperm formation

In a series of investigations the muscles of the uterine wall and in the tube have been studied during various phases of the menstrual cycle and during pregnancy. Of special interest is the discovery that there is a sphincter like type of contraction around the uterine ostia which lacks an anatomically established foundation for a sphincter effect. In close connection with these investigations the effect of various compounds on the motility in pregnant and non pregnant uterus as well as in tubes has been registered thereby adding to our knowledge of the therapeutic value of these compounds under different conditions

Ingelman Sundberg's name is widely known among gynecologists all over the world especially through his active engagement in international collaboration. For a number of years he has been co-operating in the International Federation of Gynaecology and Obstetrics (FIGO) and for several years belonged to their Executive Board representing the Scandinavian countries besides serving for a period as their Vice President. He has now been given the important mission of serving as Chairman for the FIGO Program Committee which has been allotted

the responsible task of organizing the scientific activities at the Ninth World Congress of Gynaecology and Obstetrics in Tokyo 1979

He has also engaged energetically in the publication of journals mainly as editor or co-editor for instance of *Acta Obstetricia et Gynecologica Scandinavica* *International Journal of Fertility* and *International Journal of Gynaecology and Obstetrics*. Ingelman Sundberg's great organizing and administrative ability has appeared to advantage here and under his leadership the journals have developed very favourably.

For Swedish gynecology too he has found time for stimulating work: especially as President of the Swedish Gynecological Society and in its union organization the Swedish Medical Association.

As an adviser to young clinical scientists and as a teacher of future physicians his time has also sufficed to attract the young generation to our subject area. A clear sign of this activity has been the long string of doctor's dissertations emanating from the Department of Obstetrics and Gynecology at the Sabbatsberg Hospital during his time as Chairman of the Clinic.

When Ingelman Sundberg now ends his career as Professor and Head of the Sabbatsberg Women's Clinic many thoughts of appreciation and gratitude will go to him from colleagues and disciples in the hope that he will still be able to make valuable contributions to our speciality. I am convinced that a great number of foreign gynecologists will likewise express their great appreciation of his international achievements.

Ulf Borell

It is a great pleasure for Danish obstetricians and gynaecologists to honour Axel Ingelman Sundberg on his retirement from the Chair of Obstetrics and Gynaecology at the University of Stockholm and the position as head of staff at the Sabbatsberg sjukhus.

It is not our task to praise Ingelman Sundberg's great importance within his sphere in Sweden. Our tribute is paid to him as the excellent chief editor of *Acta Obstetricia et Gynecologica Scandinavica*. His predecessor as editor-in-chief Professor Alf Sjövall had succeeded in placing the periodical on a very high level and it is Ingelman Sundberg's merit that during his editorship the journal acquired a still firmer foundation and its quality and world wide distribution were not only consolidated but also improved and increased to the benefit of Scandinavian obstetrics and gynaecology.

Danish colleagues are also indebted to Ingelman Sundberg for his efforts to

spread knowledge of Scandinavian research and attitudes especially within the International Federation of Gynaecology and Obstetrics

During his term of office Ingelman Sundberg has received many Danish colleagues in the clinic and laboratories of the Sabbatsberg Sjukhus and many more have benefited from his papers read at Scandinavian congresses where colleagues had the chance of getting to know him personally and many have become his friends. We are all united in thanking him

Mogens Ingerslev Dyre Trolle

The International Federation of Gynaecology and Obstetrics (FIGO) was founded in 1954 and since then many Scandinavian gynecologists have participated in the work of the Executive Board and the special committees of FIGO. Of these eminent gynecologists Axel Ingelman Sundberg has of course been the most active and most appreciated by FIGO. Having participated successfully in the work of both the Executive Board and the special committees of FIGO he was elected vice president of the federation during its VIIth World Congress in Moscow in 1973. In Bombay in 1975 he was also appointed associate editor of the *International Journal of Gynaecology and Obstetrics* which is the Official journal of FIGO. During the VIIth World Congress in Mexico in 1976 Axel Ingelman Sundberg was appointed chairman of the committee preparing the scientific program for the 1979 congress in Tokyo.

Twenty years have passed since the foundation of the Federation and FIGO is now firmly established. Even though it is a non governmental organization with slender means its influence is increasing. According to the first president and eminent general secretary of FIGO Professor Hubert de Watteville it is only through active co operation between the Executive Board and all the affiliated societies that the aims of FIGO can be reached. Axel Ingelman Sundberg has been and still is a very valuable and active officer of FIGO who has both kept Scandinavian gynecological associations informed of the main activities of the Federation and provided FIGO with information about scientific activities in Scandinavia that are suitable for the Federation's scientific program. For instance Scandinavians have been much more numerous as main lecturers at FIGO World Congresses than one would expect from the number of gynecologists in Scandinavia. This of course is due mainly to the high scientific standard of Scandinavian papers which is generally known but the active participation of Axel Ingelman Sundberg as a FIGO officer has certainly spread the knowledge of

Scandinavian scientists among the scientific committees of FIGO. All the friends of Axel Ingelman Sundberg here in Scandinavia are very happy that he will be continuing his activities in FIGO after retiring from his chair. His vast experience, scientific mind and good administrative judgement are needed to keep Scandinavian gynecologists and their scientific work as well known in FIGO as they are now.

Lauri Rauramo

Acta Obstetricia et Gynecologica Scandinavica has been a major scientific forum for gynecologists in the Nordic Countries for more than half a century.

Acta's fame and reputation, as well as its circulation, have continued to increase through the decades, and it has by now attained great respect within our specialty throughout the world.

Acta has been blessed by good fortune right from the beginning. The chief editors have come from the ranks of the best scientists within our special field, well known within the Nordic Countries as well as internationally.

It has been the responsibility of the chief editors to raise *Acta's* standards, to mould and to adapt it to the demands of each period.

Now that Professor Axel Ingelman Sundberg is resigning from his post as chief editor of *Acta Obstetricia et Gynecologica Scandinavica*, it is my pleasure on behalf of gynecologists in Iceland to express our gratitude for the many years of excellent and beneficial service he has performed in the interests of the field of gynecology, as well as for his outstanding administrative capabilities.

Gunnlaugur Snædal

Due to fruitful work in a wide spectrum of the medical field in his own country, in joint Scandinavian collaboration and at an international level, Axel Ingelman Sundberg has occupied a central position among Scandinavian gynaecologists for years. His achievements in Swedish medicine will be covered elsewhere. I would like to add that his initiation of gynaecological health control and of organized pediatric service at the obstetrical departments in Sweden deserves general recognition.

Axel Ingelman Sundberg was first known among his Scandinavian colleagues through his steadily increasing number of papers of common clinical and scientific

interest published in the Scandinavian journals *Nordisk Medicin* and *Acta Obstetricia et Gynecologica Scandinavica*

As a member of the Scandinavian Association of Obstetricians and Gynaecologists from 1950 he has been an unfailing attendant and a highly active participant at the congress meetings. From 1950 to 1960 his name appears fifteen times among contributors to the official transactions of the Association. The characteristic feature of his medical activity has been the broad spectrum of his interests. He has made significant contributions to *Acta Physiologica* (1) *Acta Endocrinologica* (5) *Acta Radiologica* (2) *Acta Chirurgica* (4) and *Acta Obstetricia et Gynecologica Scandinavica* (30) frequently working in close collaboration with experts in other fields.

Behind his often very sophisticated research work we can trace the solid foundation of a devoted and experienced clinician, a hall mark which has always been highly appreciated in Scandinavian medicine.

In 1968 he served as president of the Scandinavian Association of Obstetricians and Gynaecologists in Stockholm.

His achievements in the urological field of interest for obstetricians and gynaecologists are of great theoretical and practical importance, a well recognized Scandinavian contribution to international medicine.

A great deal of his research activity deals with basic problems in fertility and it was only natural that he was one of the founders of the Scandinavian Association for Studies in Fertility (1959) designed as a meeting place for physicians, veterinarians and others interested in reproductive problems. This has proved to be a very vital organization indeed with Axel Ingelman Sundberg as an unfailing and enthusiastic participant. He served as president in 1961 and 1974 and was recently elected an honorary member of the Association.

Axel Ingelman Sundberg succeeded Alf Sjövall as chief editor of *Acta Obstetricia et Gynecologica Scandinavica* on the 1st of July 1970 and resigned on the 1st of April 1977. This has been a period of accelerating research activity characterized by new techniques and new methods of approaching the problems. The period has placed heavy demands on the chief editor who has the main responsibility for preserving the tradition of our *Acta* as a highly respected and trustworthy medical journal. Axel Ingelman Sundberg had the necessary scientific qualifications, the ability to analyse scientific data critically and the sound medical judgement to meet the challenge, and *Acta Obstetricia et Gynecologica Scandinavica* is still one of the leading medical journals on clinical research. Another main editor problem during this period has been the vast number of papers presented for publication. In his unique position as editor of the *Acta* and associate editor both of the *International Journal of Gynaecology and Obstetrics* and of the *International Journal of Fertility* and as editorial consultant of *Medical*

Gynaecology Andrology and Sociology Axel Ingelman Sundberg has rendered Scandinavian research workers a valuable service by providing access to these journals and strengthening the possibility of a Scandinavian impact on international medicine. The acceptance of a small group of excellent papers from other countries in return has underlined the high quality of the *Acta*.

The improvement in typographical standards and the establishment of a sound financial basis for *Acta* also deserve to be mentioned among the achievements which should be credited the organizing and administrative talents of Axel Ingelman Sundberg.

We can safely conclude that his leadership of *Acta* has served our international reputation in a highly successful way.

This tribute to Axel Ingelman Sundberg would be very incomplete without any reference to his colourful personal qualities. His Scandinavian colleagues know that his resignation from the posts of chairman and chief editor does not mean that his vitality has been reduced only redirected. We wish him luck in his future Scandinavian and international engagements.

Oddmund Koller

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We wish to express our gratitude
to

Professor Axel Ingelman-Sundberg

for his kind and constructive cooperation over the years

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A SCHEME OF PREGNANCY MANAGEMENT

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his management of pregnancy the obstetrician knows about the condition of the fetus and individual factors influence his judgment. Nevertheless it may be useful to have some basic rules about the use of available tests (1).

Our knowledge of reproductive physiology has not been thoroughly tested by the use of these diagnostic tests and found to be satisfactory enough.

A scheme of management must be rational but simple: it must be able to be carried out in less well-equipped hospitals and it must take all pregnancies into account, not only the ones which according to often arbitrary criteria are considered high risk pregnancies. Three different steps are provided for: outpatient screening, short term hospital check-up and long term hospital evaluation and treatment.

The different parts of the scheme will be dealt with one by one.

FETAL GROWTH ASSESSMENT

Supplemented with placental function evaluation, this is the main point of the scheme. The clinical assessment of fetal growth by periodic measurement of the fundal height is at least for the time being frequently reliable. Repeated ultrasonic screening of all pregnancies is at present practically impossible.

The ultrasonic assessment will therefore be reserved only for:

- 1) The cases in which the maternal condition or any actual disease leads one to expect the theoretical possibility of poor fetal growth.
- 2) The cases in which the clinical assessment of fetal growth is uncertain.

3) The cases in which poor fetal growth has already been clinically diagnosed.

4) The cases in which poor fetal growth has already been diagnosed both clinically and ultrasonically and in which the growth rate is to be followed particularly during and after treatment.

As for the ultrasonic measurements to be chosen, we would suggest both the biparietal diameter (2) and trunk (3) (chest or abdomen) (4) circumference and the assessment of their growth rate in order (a) to see whether poor fetal growth is proportionate or disproportionate and (b) to follow the patterns of the two different measurements both for prognosis and for judging the effects of treatment.

Generally the time for ultrasonic assessment will be imposed by different conditions and circumstances rather than chosen. Of course it should be as early as possible, considering that the most convenient time for measuring the diameter and for estimating its growth rate is maintained to be between the 20th and 30th week.

ESTABLISHMENT
OF GESTATIONAL AGE

This problem is often connected with that of fetal growth. In fact, apart from the cases in which gestational age cannot be determined on the basis of menstrual history alone (about 15-22%), there are cases in which the clinical and ultrasonic assessment have provided values lower than expected, so that the alternatives are either real poor fetal growth or wrong gestational age (5).

Gestational age can be determined by comparing the ultrasonically measured diameter with the figures shown in the well known intrauterine growth charts (6). For this purpose the ultrasonic predic-

tion of gestational age by measuring biparietal diameter is best accomplished between the 20th and 30th week and is still valid between the 30th and 34th. Later on the growth rate is slower, the variability of results is increased and therefore the test becomes much less valuable.

We have already mentioned that even when one knows or believes one knows the gestational age, if values much lower than expected are obtained it is necessary to confirm whether it is a problem of incorrect gestational age determination or poor fetal growth. To this end one can resort to a comparison between two ultrasonic measurements obtained one or two weeks apart from each other, then comparing the weekly and the expected growth rate. If the two correspond a mistake in determining gestational age will be proved. If the weekly growth rate turns out to be less than expected, poor fetal growth will be assumed (7). Proper charts are available for this specific growth rate. For this purpose assessment before the 30th week is also recommended.

PLACENTAL FUNCTION EVALUATION

Coupled with fetal growth rate, placental function evaluation is the basis of the scheme. Among the different available tests, the most useful are hormonal assays and antepartum cardiotocography.

Placental function can be ascertained both by basic and dynamic tests.

(a) Basic tests

The first assessment of basic placental function in 1 pregnancies is accomplished through weekly estrogen determination (HCS is seldom used). Plasma estriol assay (8) is very helpful in some cases; nevertheless practical problems are to be expected like the refusal of the patient to provide weekly blood samples, her annoyance about reaching the clinic (sometimes far away) at fixed times and waiting for her turn and difficulties in organizing the venepuncture service which is often very busy.

Despite some objections about renal clearance (9), postural variations (10, 11) and circadian rhythm (12, 13) we believe that the urinary estrogen determination is suitable for practical purposes.

From our experience (14, 15, 16, 17, 18) with more than 9000 assays on 2½ hour urinary samples estimating the estrogen/creatinine ratio we can confirm the reliability of this method. Should we

think of changing anything, this would not be biological medium but the analytical procedure. In fact the spectrographic method (littrich) employing the Allen equation involves pigment interference and the smaller the quantities of the estrogens the more and more distorted are the results.

Furthermore analytical procedures like hydrolysis, extraction and purification bring about an estimated loss up to 50%. This is why for a long time we have been employing the radioimmunoassay using Davis Lonaux's (19) antiserum for Estriol 16 glycuronate in urinary samples, without any need of preliminary hydrolysis (20).

The weekly determination is performed starting from the 24th week. The values are expressed as an index taking gestational age into consideration. Index values below -10 are regarded as abnormal. Persistently low values or precipitous falls of 25% require admission to hospital for diagnosis and therapeutic measures.

Strict monitoring is accomplished by daily urinary estrogen assays on hospital patients. This procedure using short collection allows us to repeat the determination many times in the same day (theoretically 9 times in 24 hours) and in control within 6 hours whether the falling levels persist. This is particularly important considering the wide daily variation of estrogen level.

(b) Dynamic tests

Dynamic placental tests are very valuable although there are still considerable doubts about how well they work (22, 23). They include:

1) Estrogen gain after 17 beta-estradiol gain after DHA S load to the mother or by the intramammary route (24, 25, 26, 27).

2) Estrogen gain after progesterone administration (28, 29, 30).

3) Antepartum cardiotocography, fetal heart rate monitoring (31), step test (32), oxytocin challenge test (33).

4) Fetal electrocardiography (34, 35).

The results of antepartum cardiotocography are scored employing a suitable system (36). The dynamic tests are performed on hospital patients. They are selected and compared with one another. The hormonal ones are usually done after 3 days of bed rest. Usually they are not repeated. Cardiotocographic tests on the other hand are usually done weekly.

FETAL MATURITY ASSESSMENT

erous valid methods for the biochemical assessment of fetal pulmonary maturity are available today. Among the different procedures like thin layer chromatography (37) Clement test (38) (shake or L/S ratio) total phospholipide phosphorus assay (39) lecithin assay (40) we have adopted the first (L/S ratio) following Gluck's original method (41). According to Dunn & Bahtnagar (41) L/S ratio greater than 2 are defined as mature, those of less immature ratios between 1 and 2 are defined as 'transitional'. Using this method we always achieved a good agreement with the neonatal outcome as far as the risk of respiratory distress is concerned (42). On the basis of our own experience with cases with delayed lung maturity even at the 38th and 39th week, particularly when maternal diseases like diabetes are present, we extend the practice of pulmonary maturity assessment to all the cases that are to undergo planned delivery (43).

TREATMENT OF FETO-PLACENTAL INSUFFICIENCY

With the above combination of clinical ultrasonographic and cardiotocographic methods we reach a diagnosis of the feto-placental status. If this is impaired treatment becomes necessary. Although the results of fetal treatment in utero are promising as described in the International Parma Symposium on the therapy of feto-placental insufficiency (44), we believe that the best policy is still to deliver the fetus as soon as possible. Nevertheless, in the gestational age or fetal maturity tests especially those concerning lung maturity indicate a serious neonatal risk, there is no alternative to fetal treatment. By this we mean drug treatment improving placental blood flow (45), i.e. vasodilators (46), tocolytic (48, 49, 50, 51) or antithrombotic agents (52, 53, 54) as well as bed rest and the treatments which are required for any maternal disease. At present another possibility is to accelerate fetal lung maturity by administering glucocorticoids at least 74 hours prior to delivery (55, 56, 57, 59). The total dose is around 36 mg of dexamethasone given in 3-5 days or all at once. After the 37th week the administration can precede the pulmonary maturity test, hence the glucocorticoid will be given before amniocentesis.

PLANNED DELIVERY

This is reserved for cases showing precipitous falls in the estrogen level or impaired dynamic tests always after assessing fetal lung maturity.

However, even cases with quite normal fetal condition but with an immediate or assumed high risk can undergo planned delivery of course after checking the fetal lung maturity. Planned delivery can be vaginal or abdominal depending on the indications or the clinical findings or the results of dynamic placental tests (especially the oxytocin challenge test). Recently our cesarean section rate has been fairly high, adding those with planned delivery to those with traditional indications it is over 16%.

Thanks to a better experience, especially with antepartum and intrapartum cardiotocography, we think we may be able in the future to reduce this percentage.

INTRAPARTUM FETAL HEART RATE AND ACID-BASE BALANCE

It would be convenient for intrapartum cardiotocography to be extended to every case, even if normal. However, it is essential at least in those included in the group of planned vaginal delivery and when the above scheme has showed a condition of fetal risk (60, 61). In these cases labor will be managed by assessing acid base balance (62).

PROCEDURE

The two most important criteria assessed in this scheme are fetal growth rate and placental function (Fig. 1).

Usually the former has been assessed clinically by determining uterine size at the first consultation during the 1st trimester and then estimating growth at intervals. The hormonal evaluation of placental function by urinary estrogen assay is theoretically useful only from the 24th-26th week onwards.

At this stage of pregnancy possible doubts about gestational age should already have been removed and any poor fetal growth diagnosed, even if growth defects are more frequently evident during the third trimester. Cases of poor fetal growth as well as those with impaired placental function are bound to undergo dynamic tests and strict monitoring and they will therefore be in hospital. The same proce-

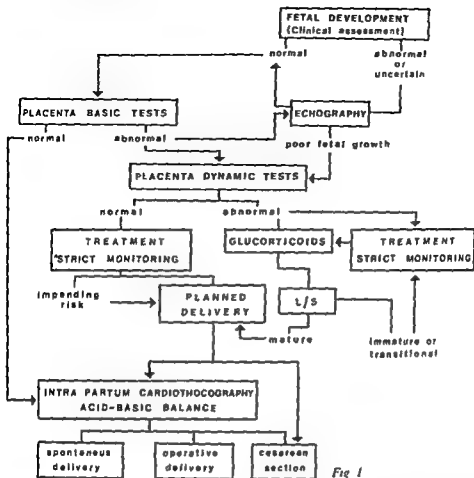


Fig 1

cedure is used in the so called high risk cases whether the risk is estimated from the history or from the mothers present condition. Cases with reassuring dynamic tests and monitoring data will be able to go as far as term or planned delivery.

Those showing very abnormal dynamic tests or a precipitous fall in the course of strict estrogen monitoring must undergo planned delivery more or less quickly according to the circumstances. At this moment gestational age will play a role of great importance in the choice of method for delivery. We suggest the utmost care when estimating fetal maturity: better not to rely upon gestational age only! From the 32nd week the amniotic fluid L/S ratio should be calculated at least 24 hours after dexamethasone administration. If the baby is judged to be mature, delivery will be planned. The most difficult problems to be resolved are cases with an impending risk and fetal immaturity. We have always continued the treatment of placental insufficiency until adequate fetal lung maturity has been attained. This policy has never resulted in

fetal death so far, and at the same time we have observed any respiratory distress in the babies born by planned delivery. Obviously this is better than others of the same kind can be labelled as too late and at the same time defective. We think that experienced and sensible obstetricians can apply and verify its real usefulness. We hope that a larger number of hospitals and centers will be able to organize and maintain a scheme of management like the one we have described.

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PREGNANCY INDUCED HYPERTENSION

1 Role of Sympathetic Nervous System and Adrenal Gland

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lateral adrenalectomy in the female results in a decrease in the urinary excretion of epinephrine by 80% and no change in the excretion of norepinephrine (1). It can be assumed that the urinary excretion of epinephrine is a relative measure of adrenal secretion of this neurohormone and the excretion of norepinephrine is primarily a reflection of sympathetic nervous system activity. Levels of epinephrine and norepinephrine can be altered by physical activity and the differences in excretion are in relation to the activity of the patient, i.e. bed rest, moderate activity or strenuous activity. Additionally, diurnal variations in excretion of epinephrine and norepinephrine also occur with a decline in both at night (2, 3).

Previous studies from my laboratories have shown that urinary catecholamine excretion can be used as an indicator of stress in pregnancy. Despite the physiologic hormonal, biochemical and anatomic changes that occur in pregnancy it has been shown that alterations in epinephrine and norepinephrine are not stressful antepartum. These studies were done in ideal conditions utilizing the same group of patients over a protracted period of time antepartum, intrapartum and postpartum for as long as consecutive months. The values of epinephrine were similar antepartum and during labor and postpartum. However, the only positive finding was an increased excretion of norepinephrine the day following delivery (4). These findings have been confirmed by Goodall & Diddle (5) who concluded that normal pregnancy and labor have no significant effect upon the adrenal medulla in respect to epinephrine and norepinephrine (5).

This report assimilates the past data that I have published as well as that of others in identifying that the adrenal gland and sympathetic nervous system are altered in toxemia of pregnancy known as pregnancy induced hypertension or preeclampsia.

MATERIAL AND METHODS

The literature has been surveyed pertaining to other studies that have identified epinephrine and norepinephrine in comparing normal pregnancy to preeclampsia. Only those studies in which individuals were studied in

normal pregnancy and preeclampsia will be included in this report since isolated studies of preeclampsia alone are of less value.

Analysis for epinephrine and norepinephrine

The method used in my laboratory to determine free urinary epinephrine and norepinephrine has been previously described (6). The method utilizes fluorometry in the trihydroxyindole procedure which is automated and measures principally epinephrine at pH 3.5 and only norepinephrine at pH 6.5. Over the years this method has been modified and now includes a radioactive tracer for sample column recovery with all results corrected to 100% to account for column loss. The urine is deproteinized with perchloric acid and the free urinary epinephrine and norepinephrine are absorbed onto alumina and later eluted with a weak acid. The eluate sample which is approximately pH 3 is adjusted to pH 6 for thioglycolic acid to determine norepinephrine or to pH 3.5 for use with ascorbic acid. The automated method requires a sampler proportioning pump system, fluorometer and sensitive recorder to measure fluorescence. Results are calculated and are usually expressed in micrograms of epinephrine and norepinephrine for 24 hours or in micrograms per hour.

DISCUSSION

The human adrenal medulla contains both epinephrine and norepinephrine of which 90% is epinephrine. Norepinephrine is the neurohormone of the sympathetic nervous system. Both of these hormones are excreted in the urine in quantities that parallel the sympatho-adrenal medullary activity. Normally during a 24 hour period the urinary excretion of epinephrine is less than 20 micrograms and usually approaches 10 micrograms. Norepi-

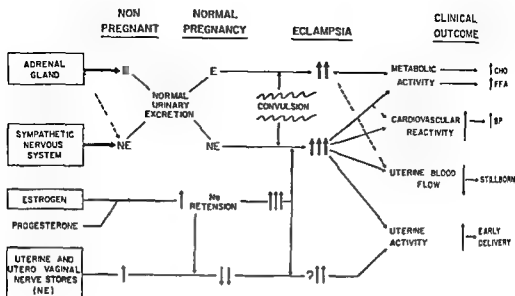


Fig 1 Interaction between adrenal gland sympathetic nervous system ovarian hormones and different uterine

nerves in the pregnant and non pregnant patient. Zupan F P. Am J Obstet Gynecol 114 304 1977

noradrenaline during this same period of time is less than 50 micrograms. The quantities of urinary excretion of epinephrine and norepinephrine during pregnancy compared to the non pregnant state is similar in the antepartum period.

Studies in the past have often been done on randomized urine samples in different patients; however, sequential studies on the same patient under similar conditions decreases the tendency for individual variation in urinary amines. These studies on the same group of patients under similar controlled environmental conditions were done to establish baseline values for normal pregnancy. These findings essentially show no change between the pregnant and non pregnant state during the antepartum or the postpartum period for either epinephrine or norepinephrine except for an increase in norepinephrine in the first 24 hours postpartum indicating late labor and delivery activity.

Preeclampsia, pregnancy induced hypertension or toxemia are terms that are used interchangeably. This broad clinical spectrum of unknown etiology makes it difficult to compare one patient to another and even more difficult to compare one investigator's patients to another investigator's patients. This clinical disease spectrum helps identify some of the confusion and occasional contradictory findings seen in this disease.

The sympathetic innervation of the reproductive tract demonstrates two types of nerves. One is a

long adrenergic neuron that originates from a paravertebral ganglion. The second type of short adrenergic neurons originate in the immediate vicinity of the effector organ (7). It has been demonstrated in the guinea pig that a decreased adrenergic transmitter in uterine nerves is seen in late pregnancy (8). The question that has not been answered is what happens to these nerves in the human in late pregnancy. More particularly, what happens to them in pregnancy complicated by hypertension and even more particularly in eclampsia. Animal studies cannot answer this question since no complete animal model exists for pregnancy induced hypertension.

It is known that catecholamines have a metabolic, cardiovascular and local effect upon the uterus as well as upon other systemic organs in the body. Norepinephrine produces peripheral vasoconstriction and additionally has an oxytocic effect upon the gravid uterus (9). Zupan (10) has proposed a schematic interaction between the adrenal gland, sympathetic nervous system with the effect of ovarian hormones, a type of uterine and uterovaginal nerves in the pregnant, normal pregnant and eclampsia state. This schema emphasizes the clinical outcome seen in the most severe of acute pregnancy induced hypertensive disease, eclampsia. He has demonstrated a marked increase in both urinary epinephrine and norepinephrine that occurs on the day of

PREGNANCY INDUCED HYPERTENSION



Proposed relationship between sodium balance pressure and neurotransmitter (NE)

convulsion. The increase persists after the convulsion and is often enhanced by delivery when it is close to the time of the convulsion. There are some epinephrine and norepinephrine values in 14 eclamptic patients in which the levels are reached that seen in a pheochromocytoma. No such reports are in the literature (10).

One issue of concern in patients that have pregnancy induced hypertension is to consider the role of epinephrine and norepinephrine in relation to changes in cardiovascular reactivity. We have studied the cardiovascular sensitivity and reactivity to infusions of angiotensin. Norepinephrine and epinephrine demonstrate hyperreactivity in patients that have preeclampsia (11). It was postulated at the time that the increased sodium in the vessel wall may account for the altered vascular reactivity. This area needs further investigation in trying to demonstrate the relationship between sodium balance, blood pressure, and the neurotransmitter epinephrine (11). There is a decreased ability of sympathetic granules to bind and store norepinephrine when sodium is in excess (Sodium is in excess in preeclampsia). If there is reduced capacity for binding and storage, this might make more available free circulating norepinephrine. The patient's own amines could react on receptor sites and increase the cardiovascular reactivity. This could be as a positive feedback mechanism for the development of hypertension (Fig 2).

In addition to the above study, Zuspan, Nelson & Ahlquist (17) demonstrated an increased cardiovascular reactivity to an infusion of epinephrine in mild preeclampsia (Fig 3). The question as to whether or not milder forms than eclampsia will be associated with the disruption of homeostasis of the neurotransmitters seen in normal pregnancy needs further

Table I

	Epinephrine (μg/h)	Norepinephrine (μg/h)
Normal antepartum (balance ward hospitalized)	0.21	1.16
Controls		
1st 17 hours hospitalized	0.97	0.84
2nd 17 hours hospitalized	0.43	1.45
3rd 17 hours hospitalized	0.51	0.64
Mean	0.64	0.98
Pregnancy induced hypertension moderate severity		
1st 17 hours hospitalized	1.97	1.64
2nd 17 hours hospitalized	1.68	1.72
3rd 17 hours hospitalized	1.31	1.49
Mean	1.65	1.62

clarification. Urinary amine excretion during pregnancy has been investigated by Pekkarinen & Castren on 31 patients with severe preeclampsia compared to 310 normal patients utilizing the measurement of urinary vanilmandelic acid (VMA). There was no difference in the groups' excretion of this substance and it was concluded that this was an insensitive endpoint measurement between the two groups (13).

Research has demonstrated that urinary levels of epinephrine, norepinephrine, and vanilmandelic acid are significantly higher in severe preeclampsia than in normal pregnant women and that the magnitude of response was somewhat proportional to the severity of the disease (14). Zuspan has confirmed

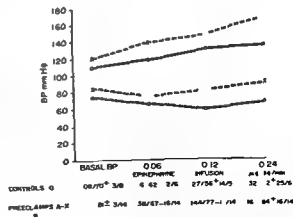


Fig 3 Data modified from previous study of Zuspan et al demonstrating altered cardiovascular reactivity to epinephrine (Zuspan F, Nelson G H & Ahlquist R. *Am J Obstet Gynecol* 90:97, 1964).

Table II Cardiovascular reactivity

	Response to infusion of	
	Epinephrine	Norepinephrine
Non pregnant	+	+
Normal pregnant	+	+
Preeclampsia	++	+++

these findings in which controlled patients were compared to preeclamptic patients when they are first admitted to the hospital for 12 hour periods of observation. Table I illustrates that in normal antepartum patients under balance ward conditions the epinephrine was 0.21 micrograms per hour contrasted to controls in the study mentioned above of 0.64 which is compared to moderately severe pregnancy induced hypertension of 1.65. The norepinephrine values also show a similar trend in that normal antepartum (balance ward) was 1.16 the controlled study was 0.98 and the patients with pregnancy induced hypertension was 1.62 (15).

The amount of vascular reactivity correlates well with the severity of pregnancy induced hypertension (Table II). The increased amine activities in pregnancy induced hypertension may be a factor that aggravates the disease. It is impossible to state whether or not this is a cause-and-effect relationship since many co variables alter neurohumoral mechanisms. To date no one has identified the reason for the increased amine excretion since it could be from changes in metabolism, binding receptor activity and sodium balance. We are left with knowledge that there is increased amounts of epinephrine and norepinephrine associated with pregnancy induced hypertension. Their role to date is yet to be determined.

ACKNOWLEDGEMENT

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COMPARATIVE STUDY OF UTERO INHIBITING ACTION OF TWO β ADRENOMIMETIC DRUGS

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Gynecologist and Obstetrician frequently encounter problems in which the health of the fetus is threatened by uterine activity. Such uterine contractions occasionally occur after surgical intervention in which the pelvic viscera have been manipulated, or because during labour one or several of the components of uterine activity are abnormally excited or when uterine contractility with normal characteristics interferes with the placental function.

The necessity of decreasing the uterine contraction when it threatens the wellbeing of the fetus has led to the search for a pharmacological agent which offers the greatest uterine inhibiting action with the fewest undesirable secondary effects. The best prospect lies in the use of β adreno-mimetic drugs. Among these, the one which has been best studied in the Hospital de Gineco-Obstetricia No. Uno del Instituto Mexicano del Seguro Social is orciprenaline (4, 5, 6). Although this is not the ideal uterine inhibiting drug, it is known to us, and as such has been taken as the parameter of comparison for the other β -adreno-mimetic agents studied.

In this paper we present three results of the comparison between Th 1165 and orciprenaline.

Orciprenaline (C. H. Boehringer Sohn, Ingelheim am Rhein, Germany) 1 (3,5-dihydroxyphenyl)-2-isopropyl aminoethanol hydrochloride.

Th 1165 (C. H. Boehringer Sohn, Ingelheim am Rhein, Germany) 3,5-dihydroxy- α [(p-hydroxy- α -methyl-phenetyl)-amino]-methyl-benzylalcohol hydrobromide.

PATIENTS AND METHODS OF STUDY

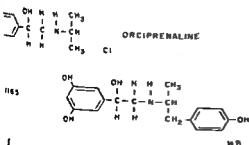
To date thirty-seven patients have been selected for study (17 for Th 1165) from the admission service of the Hospital de Gineco-Obstetricia No. Uno. Patients have been selected to meet the following criteria:

- (a) Term pregnancy (38 to 42 weeks of amenorrhea)
- (b) Systolic blood pressure between 100 and 140 mmHg and the diastolic blood pressure between 60 and 90 mmHg
- (c) Parity greater than 1 and less than 4
- (d) No clinical manifestation of maternal or fetal pathology or cephalopelvic disproportion
- (e) Early labour with cervical dilatation greater than 2 cm and less than 5 cm
- (f) Membranes intact

In these patients continuous recording of intra-amniotic pressure was made following the technique of Caldeyro-Barcia (1) in order to quantify the modifications of uterine activity.

Continuous recordings were also made of arterial pressure through a polyethylene catheter inserted into the abdominal aorta via the femoral artery (6); maternal heart rate by integrating the R waves of the maternal electrocardiogram (ECG) and fetal heart rate by integrating the modification on ultrasonic waves produced by the heart beats (Doppler's effect).

Maternal and fetal ECG tracings were recorded before and after administration of β -adrenomimetic drugs. The maternal ECG's were taken with conventional electrodes and methods. The fetal ECG's were taken with a conven-



UTERINE TONUS BEFORE AND DURING INFUSION

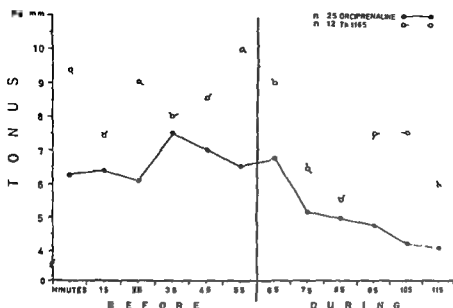


Fig 2

tional electrocardiograph using an Elemedix fetal ECG preamplifier and the scalp electrode of Hon (7).

All patients were given intravenously $0.03 \mu\text{g}$ of Th 1165a per minute per kilogram of body weight during 60 min. The Th 1165a was diluted in isotonic sodium chloride solution.

Twenty five patients selected with the same criteria were given intravenously $0.25 \mu\text{g}$ of orciprenaline per

minute per kilogram of body weight during 60 min. The orciprenaline was also diluted in isotonic sodium chloride solution.

Maternal arterial blood samples and fetal scalp capillary blood samples were obtained at the beginning and at the end of infusion for acid base balance, glucose, lactate and pyruvic estimations.

RESULTS

For each treatment the values of tonus, frequency and uterine activity were measured one hour before and one hour during the influence of the drug. These values were then analyzed.

In the statistical analysis the great variability represented by the different parameters within each patient was taken into consideration. This was done between patients of the same group and between groups.

The means for each period of 10 min before

Table I Uterine contractility. Mean values of variables one hour before and one hour during Th 1165a infusion ($n=12$)

Variable	Th 1165a (means)		P
	Before	During	
Tonus	8.74	6.99	<0.05
Intensity	76.87	17.05	<0.005
Frequency	4.61	3.99	n.s.
Uterine activity	116.65	70.70	<0.005

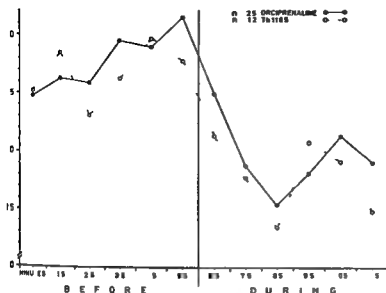
Table II Uterine contractility. Mean values of the variables one hour before and one hour during orciprenaline infusion ($n=25$)

Variable	Orciprenaline (means)		P
	Before	During	
Tonus	6.65	5.00	<0.005
Intensity	27.79	19.54	<0.005
Frequency	4.76	4.27	n.s.
Uterine activity	132.84	75.37	n.s.

Table III Uterine contractility. Comparison of variables during infusion of two drugs: orciprenaline ($n=25$) and Th 1165a ($n=12$)

Variable	Drug infusion (means)		P
	Orciprenaline	Th 1165a	
Tonus	5.00	6.99	<0.01
Intensity	19.54	17.05	n.s.
Frequency	4.77	3.99	n.s.
Uterine activity	75.37	70.70	n.s.

ACTIVITY OF UTERINE CONTRACTIONS BEFORE AND DURING INFUSION



UTERINE ACTIVITY BEFORE AND DURING INFUSION

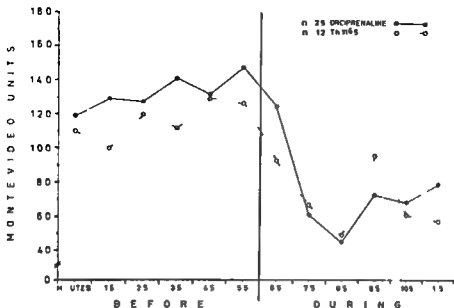


Fig 5

increased before the administration of the drug but decreased to 40% of its average value during infusion (Fig 5)

The difference is significant at a level of 0.005. There was no difference between the two drugs.

The maximum action of orciprenaline on uterine contractility was reached at the 25th minute of infusion. The largest uterine inhibitory effect with Th 1165 was reached at the 15th minute.

Apparently the decrease of uterine activity with the studied drugs corresponds to the decrease of the intensity, since the frequency was not significantly modified with its administration. The acid base balance in patients under Th 1165 infusion were similar to those found in patients receiving orciprenaline (2).

DISCUSSION

Our results for orciprenaline are in agreement with those reported by Poseiro et al (6) and Eskes et al (7). The data published by Gamissans et al (8) for Th 1165 are similar to our results.

The comparison of variables in uterine contractility with the above drugs given intravenously showed no statistical difference except for the tonus. This could be due to the smaller number of cases studied with Th 1165.

Both drugs diminish uterine activity by reducing the intensity of contractions.

It can be observed that Th 1165 has a milder and more persistent action than that of orciprenaline during the hour of infusion. Nevertheless a larger number of cases has to be studied to draw definite conclusions.

Secondary effects on the mother such as arterial hypotension and tachycardia were less marked with Th 1165 than in the orciprenaline series.

The fetal tachycardia was also less severe if the mother was given Th 1165 than if she received orciprenaline.

Among the uterine inhibitory drugs the β -adrenomimetic agents seem to have a very promising future.

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 **FERROSAN**

THE EFFECT OF SALBUTAMOL AND TERBUTALINE IN THE MANAGEMENT OF PREMATURE LABOUR

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Abstract A comparative study concerning the effect of salbutamol and terbutaline in the management of premature labour with intact membranes has been performed. A group included 34 patients between 27-36 weeks gestation. The drugs used were given by intravenous infusion. Salbutamol arrested labour activity in 33 of 34 patients and terbutaline in 32 of 34 patients. Delivery was postponed for more than one week in 74% of the patients receiving salbutamol and in 80% of the patients receiving terbutaline. No significant difference in effect was found between the drugs used. The effect was equally good independent of the gestational age. Dilatation of cervix of 2 cm was associated with successful treatment (delay of delivery more than one week) in 27% compared with 14% if cervix was dilated 1 cm or less. In 12 patients with ruptured membranes a temporary arrest of labour activity (1-7 days) was obtained in 7 patients and in 1 patient the delivery was delayed for 6 weeks. In the doses used an increase in maternal and fetal heart rate was observed more frequently in the patients receiving salbutamol compared with those receiving terbutaline. No serious side-effects were observed neither in the salbutamol nor the terbutaline group. It is concluded that these drugs are well tolerated and effective in the treatment of premature labour.

The efficiency of β receptor stimulating adrenergic drugs to inhibit uterine contractions is well documented. The first clinical report concerning the efficacy of these drugs in inhibiting uterine activity was published by Hendricks et al. (6). Clinical usefulness was however limited due to adverse side-effects, i.e. tachycardia, palpitations and tremor. In recent years drugs with more selective stimulatory effect on the β receptors which are responsible for the inhibitory effects on uterine contractions have become available. Among these drugs terbutaline has been shown to inhibit uterine activity (1) and prevent premature delivery (7). Re-

cent studies measuring the relaxing effect upon the pregnant human uterus *in vivo* have shown that another adrenergic β receptor agonist salbutamol was more effective than terbutaline in this respect (2). Clinical trials have also demonstrated that salbutamol is effective in inhibiting uterine contractions (10) and preventing premature delivery (9).

No comparative study concerning the clinical effect of these drugs in the management of premature labour has previously been performed. The aim of the present study was to compare the effect of salbutamol and terbutaline in premature labour and to study the influence of these drugs on maternal and fetal heart rate and maternal blood pressure.

MATERIAL AND METHODS

The study was performed during the period May 1974 to April 1976. The patients included in the comparative study fulfilled the following criteria: Active uterine contractions with a frequency of at least two contractions every 10 min verified by means of external tocography over a period of about 30 min; intact fetal membranes; and a duration of pregnancy between 27 to 36 weeks. Patients with ruptured membranes, twin pregnancies or those who had been operated upon for cervical incompetence were excluded. 34 patients received salbutamol and 34 patients terbutaline by random selection. An analysis of the patients with reference to the state of the cervix, the length of pregnancy and the frequency of contractions at the start of the treatment (Table I) indicated that the groups were comparable in these respects. A statistical analysis using Student's *t* test could not demonstrate any statistical differences between the groups.

One group comprising 17 patients with ruptured membranes and labour activity have also been treated with salbutamol or terbutaline. These patients are reported separately.

The drugs were administered by intravenous infusion using an infusion-pump or a drip counter.

Table 1 Comparison of patient characteristics and treatment

	Salbutamol	Terbutaline
Number of patients	34	34
Frequency of contractions		
<3/10 min	21	24
≥3/10 min	13	10
Mean value ± S D (contractions/10 min)	3.1 ± 1.3	2.7 ± 1.1
Cervical dilatation		
≤2 cm	21	25
>2 cm	13	9
Mean value ± S D (cm)	2.0 ± 0.9	2.0 ± 0.8
Length of pregnancy		
≤30 weeks	7	5
31–33 weeks	18	13
34–36 weeks	9	16
Mean value ± S D (weeks)	32.1 ± 2.2	32.9 ± 2.0

Salbutamol was diluted in 5% dextrose to a concentration of 25 µg/ml. The initial dose was 25 µg/min. The dosage was thereafter individually adjusted depending on the maternal heart rate or uterine activity within the range 6.25–37.5 µg/min, usually between 6.25–12.5 µg/min. The treatment was continued for a varying length of time, usually between 12 to 24 hours. One patient received 160 mg salbutamol over 6 days.

Terbutaline was diluted in 5% dextrose to a concentration of 5 µg/ml. The initial dose was 5.0–7.5 µg/min. The dose was thereafter adjusted depending on the maternal heart rate or uterine activity within the range of 2.5–15 µg/min, usually between 2.5–5.0 µg/min. The treatment was continued for a varying length of time, usually be-

tween 12–24 hours. In one patient 77.5 mg terbutaline was given over 80 hours.

Maternal systolic and diastolic blood pressure and fetal heart rate were registered during the first 10 min, and thereafter at least once every 15 min.

After cessation of the infusion the treatment was continued with bed rest and oral administration of salbutamol 2 mg×4 or terbutaline 5 mg×3 respectively.

RESULTS

The results obtained are summarized in Table 2. Delivery within 24 hours occurred in one patient in the salbutamol group and in two patients in the terbutaline group. A prolongation of the gestation for at least one week occurred in the terbutaline group in 80% of cases and in the salbutamol group in 74% of cases. Most of the patients were delivered between the 37th to 39th week of pregnancy. A significant increase in maternal and fetal heart rate was obtained more frequently in the salbutamol than in the terbutaline group. No patient developed side effects which necessitated discontinuance of the treatment. The effect on blood pressure in the doses used was very small. Generally a small increase in the systolic blood pressure and a small decrease in the diastolic blood pressure were found, but the effects were not consistent.

No perinatal deaths occurred in the total study groups. Among 25 babies with gestational age less than 37 weeks there were only 7 infants who developed respiratory distress syndrome.

Table 2 Results of treatment

	Salbutamol		Terbutaline	
	n	%	n	%
Number of patients	34		34	
Prolongation of pregnancy				
≥1 week	25	74	27	80
1–7 days	8	23	5	14
<24 hours	1	3	2	6
Mean value (days) ± S D	31.3 ± 2.3		31.1 ± 0.4	
Gestational age at delivery				
<37 weeks	14	41	17	50
37–39 weeks	15	44	18	53
40–42 weeks	5	15	4	12
Mean value (weeks) ± S D	36.5 ± 3.3		37.2 ± 2.5	
Increase in maternal heart rate ≥40 beats/min	11		8	
Increase in fetal heart rate >20 beats/min or heart frequency >160 beats/min	9		3	

III Results of treatment according to the gestational age and the state of the cervix at the onset of preterm labour

	n	Prolongation of pregnancy					
		>1 week		1-7 days		<24 hours	
		n	%	n	%	n	%
Gestational age at onset of preterm labour							
<30 weeks							
0	12	8	67	4	33	0	0
33	31	5	81	4	13		6
36	5	19	6	5	0	1	4
Cervical dilatation at onset of preterm labour							
<1 cm	53	48	91	5	9	0	0
≥1 cm	15	4	27	8	53	3	20

no difference was found between the salbutamol and terbutaline group with respect to the effect on uterine activity the groups have been combined in order to analyze the influence of the state of the cervix and the duration of pregnancy at commencement of treatment on the outcome of treatment. The results are shown in Table III. Duration of pregnancy did not influence the effect of the treatment. If cervix was dilated more than 1 cm the treatment was successful (prolongation of pregnancy at least one week) in only 27% compared to 91% if cervix was dilated 2 cm or less. In 17 patients with ruptured membranes the drugs delayed labour between one and seven days in 7 patients and in 1 patient the delivery was delayed for 6 weeks.

DISCUSSION AND CONCLUSION

The present study confirms previous results (7, 9) that β_2 stimulating drugs with more selective β_2 stimulating properties are very effective in arresting premature labour without serious adverse side-effects to the mother or infant. In the present study no difference in effect could be found between the salbutamol and terbutaline treated group. In the salbutamol group a somewhat higher incidence of increased fetal heart rate was found. The explanation might be that the doses used in the two groups are not entirely comparable. This is also demonstrated by the fact that in one patient the initial salbutamol dose of 25 $\mu\text{g}/\text{min}$ was increased whereas in the terbutaline group the initial dose of 5 $\mu\text{g}/\text{min}$ was increased in 8 patients.

It is a well known fact that the diagnosis premature labour cannot easily be distinguished from

false labour. Thus the results of placebo treatment in different studies vary remarkably: 20% (7), 38% (14) and 73% (4). The different results must depend on the selection of the subject and implies that results from different studies are not comparable. With the present knowledge of the effect of β stimulating drugs on premature contractions it must however be unethical to perform a study using a placebo group. In the present study only patients with regular contractions at an interval of at least two per 10 min were included in the study. Most of the patients (87%) were delivered before 40 weeks gestation which might indicate that the patients included in the study really were in active labour.

In the present study there was no difference in effect between patients of different gestation. In a previous study (11) concerning the effect of ethanol on premature labour it was found that ethanol treatment was more effective in patients with a gestational age of 35-36 weeks (95%) in comparison to 27-32 weeks (67%). The explanation might be that different causes of premature labour are dominant at different gestations. In late pregnancy the sensitivity of the myometrium to oxytocin is increased (5). Oxytocin released from the neurohypophysis might therefore be able to induce labour contractions. Oxytocin release can be inhibited by administration of ethanol (12). At an earlier gestation other factors contribute to the induction of labour therefore ethanol is less effective in these patients whereas β stimulating adrenergic agents which act directly on the myometrial cell are equally effective at all gestations.

As regards the state of the cervix and the outcome of pregnancy it is obvious that if cervix is dilated more than two centimeters the effect of the

treatment is less reliable. Generally only a temporary arrest of labour activity is obtained. The results stress the importance of a rapid instigation of treatment to achieve still better results.

If the fetal membranes are ruptured the results of treatment with β stimulating agents are rather poor. A temporary arrest of uterine activity can however often be obtained.

A remarkable observation in the comparative study is that of the 25 infants born before 37 weeks of pregnancy only 2 developed respiratory distress syndrome (RDS) despite no treatment with glucocorticoids having been given to prevent RDS. This observation supports recent retrospective studies indicating that the incidence of RDS is greatly reduced in premature infants born of mothers treated with β mimetic drugs (3-8). Experimental studies on fetal rabbits also indicate that β adrenergic agonists may enhance the release of surface active material into the pulmonary alveoli. The present study also indicates that treatment with β stimulating agents in the doses used seems to be without risks for the fetus and mother.

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THE EFFECT OF BETA RECEPTOR STIMULATING AGENTS ON THE UTERO PLACENTAL BLOOD FLOW

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Abstract. The influence of salbutamol, a beta₂ receptor stimulating agent, on the blood flow through the uteroplacental unit was evaluated in the human. Serial placentalograms were analysed quantitatively after injection of 5 mCi Indium 113m by means of a gamma-camera connected on line to a computer. The examinations were performed in the third trimester of pregnancy. No sedation was used. Uterine contractions were not present. Salbutamol caused an increase in activity over the placental region corresponding to a 15% increase in blood volume. The rise time of the initial phase of isotope accumulation (calculated from 5 to 95% of final activity) was prolonged by 100% during salbutamol infusion. As the rise time is proportional to the volume/flow ratio of blood in the uteroplacental region, our data indicate that salbutamol infusion causes a decrease in blood flow in the face of uterine contractions.

Beta receptor stimulating agents have been employed in obstetrical practice during the last ten years. The efficacy with which these drugs decrease uterine tone in smooth muscle and arrest uterine contractions is well documented in the literature. These properties of the drugs have led to their use in premature labor and in obstetrical emergencies (10).

Beta receptor stimulating agents have been recommended to improve fetal condition, especially in the final stage of their administration and secondary to arrest of strong uterine contractions (3). The drugs have also been found of value for the treatment of fetal asphyxia (13). Little information is available, however, regarding their effect on placental blood flow and fetal oxygenation. In the pregnant ewe, isoproterenol has been reported to increase uterine blood flow in spite of a decrease in perfusion pressure (8). More recently, contrary re-

sults have been obtained in studies using other synthetic beta receptor stimulating drugs (4). Studies in sub-human primates have shown more over that the administration of isoproterenol to the sedated mother leads to a reduction of the arterial oxygen tension in the intrauterine fetus, the fetal arterial PO₂ being taken as an indicator of the perfusion of the intervillous space (7).

The aim of this investigation has been to study the influence of beta receptor stimulating agents on the blood flow through the placenta in the human. Scintigraphic techniques were chosen and two modifications have been employed in determining the amount of radioactivity over the placental site following intravenous injection of ^{113m}Indium-chloride during basic conditions and during the administration of salbutamol.

MATERIAL AND METHODS

Sixteen pregnant women in the last trimester of pregnancy, referred for scintigraphic localization of the placenta because of clinical suspicion of placenta previa, were asked to participate in this investigation, authorized by the local ethical committee. Determination of steady state of the radioactivity over the placental site was performed in 11 women and the initial phase of accumulation of radioactivity was determined in another five. For this purpose we used ^{113m}In which following its intravenous injection is bound almost completely to transferrin and only a negligible amount of activity (less than 0.5%) will pass the placental barrier to the fetus. The fetal radiation dose is only about 10 mrad per mCi given to the mother (5).

Determination at steady state of isotope uptake over the placental site was performed using a gamma-camera with diverging collimator on line with a computer positioned in a frontal view over the placenta. Serial

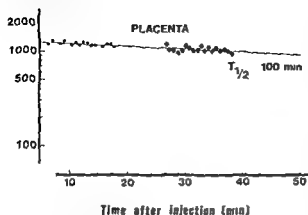
Activity
counts/40 s

Fig 1 Time function of activity in placenta after injection of ^{113m}In (subject MU)

tigrams were recorded in image matrices of 64×64 elements corresponding to a geometric resolution of about 5 mm. The measurement started a minimum of 5 min after the intravenous injection of 1 mCi of ^{113m}In thereby ensuring that the isotope and blood were homogeneously mixed before recording. The sampling time of each scintigram was 40 s and the total recording time 12 min. By computerized summation of the scintigrams a detailed image was obtained in which the placenta could be identified with accuracy and outlined for subsequent analysis of the time activity distribution.

Five of the 11 women received an intravenous infusion of salbutamol in a dose of $15 \mu\text{g}/\text{min}$ beginning after the completion of the recording of basic conditions. The radioactivity over the placental site was then recorded again at 40 s intervals during another 12 min. This recording started 5–7 min after the commencement of the salbutamol administration. Six women who did not receive salbutamol were examined during a similar additional recording period for control purposes.

Determination of initial phase of isotope accumulation over the placental site was performed in 5 women. These measurements were performed using a 50×50 mm NaI (TI) detector with a conic collimator (length 190 mm front opening 90 mm) positioned over the placenta previously localized by ultrasonography. The detector was connected to a time scaling multi-channel analyser. The recordings started in direct connection with the intravenous injection of 0.5 mCi ^{113m}In . The radioactivity was determined during 9 s periods for 6 min. Each patient was examined on two separate occasions: first during basic conditions and second during the i.v. infusion of salbutamol in a dose of $15 \mu\text{g}/\text{min}$. The administration of salbutamol was always started 15 min before the isotope injection. This latter detector system was preferred for the measurement of radioactivity accumulation as it is more sensitive than the gamma-camera. The duration of sampling times could then be minimized (9 s) without losing satisfactory statistical precision during the initial steep part of the time activity curve.

The continuous recording of arterial blood pressure and the withdrawal of blood samples at intervals for analysis of acid base parameters were facilitated by use of an intraarterial catheter inserted transcutaneously into the brachial artery of the mother before the commencement of the examinations. The blood pressure was recorded on an ink jet recorder via an inductive pressure transducer connected to this catheter.

RESULTS

Determination at steady state of isotope accumulation over the placental site. When the individual values of counts per 40 s period had been plotted along the y axis in a semilogarithmic diagram against time along the x axis, all values could be adapted to a single line with a slope representing the physical half-life of ^{113m}In . The values during the second period of determination in the women who did not receive salbutamol were in all instances positioned along the continuation of the line representing the physical half-life of the isotope, which was found to be 100 min. The period of determination in the same individual was 12 min.

1) The observations during infusion of salbutamol, however, followed a line which was displaced 1–2 times towards higher activity values but fitted the line with the line reflecting basic conditions (Fig 1).

Values from 18 points of the first 12 min period of measurement were then added and compared with the sum of the values from 18 points of the second period after correction for the physical decay. Table I the results of these two time periods are given for each control patient. There were no significant differences.

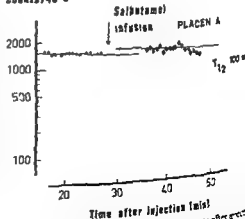
Activity
counts/40 s

Fig 2 Time function of activity in placenta after injection of ^{113m}In before (filled circles) and during $15 \mu\text{g}/\text{min}$ salbutamol infusion (subject MN)

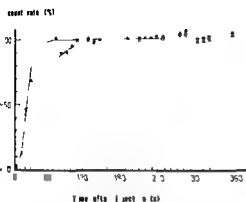


Fig. 1 Time function of initial accumulation of ^{113m}In in placenta without (filled triangles) and with (open circles) infusion of $15 \mu\text{g}/\text{min}$ salbutamol (subject BP). The curves are normalized to 100% at their respective maximum.

significant changes during these two periods, the ratio of uptake between the first and the second period being equal to one. When salbutamol was infused the infusion was given before the second period and it led to an increase in the uptake of ^{113m}In in the placental region by an average of 15%, equivalent to a 15% increase in blood volume (Table II).

Determination of initial phase of isotope accumulation over the placental site. The maximum activity registered in each patient was taken as 100% and used for the normalization of values during the initial part of the curve. It was observed that the accumulation of activity over the placental region was considerably slower during the administration of salbutamol than during basic conditions. The rise was defined as the time for increase of activity

Table I Registration of ^{113m}In steady state uptake in placenta in control patients

	Uptake in placenta (counts)		Ratio of uptake between second and first period
	First period	Second period	
1	70.7×10^3	19.8×10^3	0.98
	17.9×10^3	71.4×10^3	1.19
	15.9×10^3	17.0×10^3	1.07
1	25.0×10^3	24.4×10^3	0.98
	27.8×10^3	30.4×10^3	1.09
	8.3×10^3	77.7×10^3	0.98
1.05 ± 0.09 (1 S.D.)			

Table II Effect of salbutamol infusion on the ^{113m}In steady state uptake in placenta

Subject	Uptake in placenta (counts)		
	Reference period (first period)	Salbutamol infusion (second period)	Ratio of uptake with and without salbutamol
MN	73.6×10^3	27.1×10^3	1.15
IK	4×10^3	26.1×10^3	1.17
KN	74.9×10^3	26.6×10^3	1.07
LN	41.9×10^3	48.7×10^3	1.17
EM	40.0×10^3	46.7×10^3	1.17
Mean	1.15 ± 0.05 (1 S.D.)		

from 5–95% of the maximum count rate was prolonged 100%. The results are given in Table III and depicted in Fig. 3.

Arterial blood pressures were normal in all patients. Only small changes in arterial pressures were recorded during salbutamol infusion (Fig. 4). On average there was a slight increase in the systolic pressure while the mean and diastolic pressures were essentially unchanged. Concomitantly a progressive increase in heart rate was registered during the first 15 min of infusion of salbutamol. At the end of the infusion period heart rate had increased 40% and the women invariably felt palpitation and tremor. The arterial tensions of O_2 and CO_2 and the pH values were within the normal range and were not significantly influenced by the infusion of salbutamol.

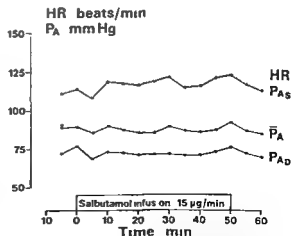


Fig. 4 Arterial blood pressure (\bar{P} : systolic, mean, diastolic) and heart rate (HR) during salbutamol infusion.

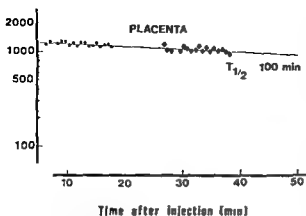
Activity
counts/40 s

Fig 1 Time function of activity in placenta after injection of ^{113m}In (subject MU)

tigrams were recorded in image matrices of 64×64 elements corresponding to a geometric resolution of about 5 mm. The measurement started a minimum of 5 min after the intravenous injection of 1 mCi of ^{113m}In , thereby ensuring that the isotope and blood were homogeneously mixed before recording. The sampling time of each scintigram was 40 s and the total recording time 12 min. By computerized summation of the scintigrams a detailed image was obtained in which the placenta could be identified with accuracy and outlined for subsequent analysis of the time activity distribution.

Five of the 11 women received an intravenous infusion of salbutamol in a dose of $15 \mu\text{g}/\text{min}$ beginning after the completion of the recording of basic conditions. The radioactivity over the placental site was then recorded again at 40 s intervals during another 12 min. This recording started 5–7 min after the commencement of the salbutamol administration. Six women who did not receive salbutamol were examined during a similar additional recording period for control purposes.

Determination of initial phase of isotope accumulation over the placental site was performed in 5 women. These measurements were performed using a 40×50 mm NaI (TI) detector with a conic collimator (length 190 mm, front opening 90 mm) positioned over the placenta previously localized by ultrasonography. The detector was connected to a time scaling multi-channel analyser. The recordings started in direct connection with the intravenous injection of 0.5 mCi ^{113m}In . The radioactivity was determined during 9 s periods for 11 min. Each patient was examined on two separate occasions: first during basic conditions and second during the iv infusion of salbutamol in a dose of $15 \mu\text{g}/\text{min}$. The administration of salbutamol was always started 15 min before the isotope injection. This latter detector system was preferred for the measurement of radioactivity accumulation as it is more sensitive than the gamma-camera. The duration of sampling times could then be minimized (9 s) without losing satisfactory statistical precision during the initial steep part of the time activity curve.

The continuous recording of arterial blood pressure, the withdrawal of blood samples at intervals for analysis of acid base parameters were facilitated by use of an intraarterial catheter inserted transcutaneously into the brachial artery of the mother before the commencement of the examinations. The blood pressure was recorded on an ink jet recorder via an inductive transducer connected to this catheter.

RESULTS

Determination at steady state of isotope activity over the placental site. When the individual values of counts per 40 s period had been plotted along the y axis in a semilogarithmic diagram and plotted along the x axis, all values could be adapted to a line with a slope representing the physical half-life of ^{113m}In . The values during the second period of determination in the women who did not receive salbutamol were in all instances positioned on the continuation of the line representing the decay of the isotope, which was found during the first period of determination in the same individual (Fig. 1).

1) The observations during infusion of salbutamol, however, followed a line which was displaced 1.5 times towards higher activity values but parallel with the line reflecting basic conditions (Fig. 2).

Values from 18 points of the first 17 min period of measurement were then added and compared with the sum of the values from 18 points of the second period after correction for the physical decay. Table I the results of these two time periods are given for each control patient. There were no

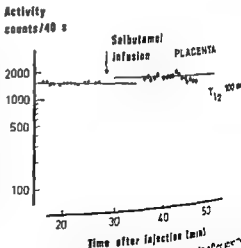


Fig 2 Time function of activity in placenta before (filled circles) and during (filled squares) salbutamol infusion (subject MN)

cular beds in the somatic vessels would be to divert blood away from the uterine circula-
 -Reflexly mediated regional vasoconstriction
 a resultant reduction in conductance not
 1 to any specific change in mean arterial pres-
 - might also occur (11) The increased rise time
 tope accumulation and the decrease in blood
 over the placental region found in this study
 be secondary phenomena induced by these
 mechanisms. Furthermore isoproterenol
 ■ the placenta readily and might cause a re-
 bution of fetal blood flow with a diversion of
 ight ventricular output of the fetus from the
 sion of the placenta as a result. More detailed
 s with refined techniques are needed in or-
 to increase our knowledge regarding the
 nacologic effects of various beta receptor
 ating drugs during pregnancy.

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THE INFLUENCE OF PRESSURE UPON THE FETAL HEAD DURING LABOUR

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act In contracted pelvis moulding of the skull is caused by the force of the amniotic fluid pressure. The resistance of the pelvis. In normal labour and normal uterine action the moulding of the skull bones is caused by the head to cervix pressure, as has been demonstrated by intra uterine tokometry. By contrast in the contracted pelvis there are no mouldings between the two fetal bones. In breech presentation during the first stage of labour the moulding of the skull bones is caused by the differing resistance of the various skull bones against the amniotic fluid pressure.

Skull lesions of the fetus caused by various causes are seen in operations such as extraction of the fetus in vertex as well as in breech presentation.

The fetal head in particular is affected by various pressures which cause moulding of the skull bones and fractures are sometimes seen. The mouldings may cause rupture of the tentorium and cerebral hemorrhage. In a conservatively treated fetal skull fracture from Sabbatsbergs hospital 1949-1959 comprising 23836 infants 647 died perinatally. Of these infants 17.3% showed rupture of tentorium (Lindgren et al 1962). By active obstetric management the risk has been reduced but we do not know how many children survived who had cerebral palsy or mental retardation caused by these skull fractures.

Skull mouldings occur as a result of contracted pelvis also in cases of normal labour, abnormal uterine action, secondary inertia and in breech presentation. In the latter cases the mouldings are caused by various intra uterine pressures and the increased resistance of the pelvic floor. The purpose of this paper is to demonstrate the various types of skull mouldings and the different pressure conditions which cause these mouldings.

METHOD

In collaboration with Ingelman Sundberg & Ljungström (5) the method of intra uterine tokometry was worked out by using strain gauges. By using this method we have been able to study the biomechanics of the cervix in labour (6, 7, 8).

The X ray pictures have been made by Ingemar Fernström M.D. Karolinska sjukhuset Stockholm.

Normal labour

By using the method of intra uterine tokometry we found that during contractions in vertex presentation the head to cervix pressure is on average three to four times higher

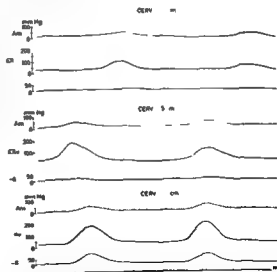


Fig 1 Recordings of the intra uterine pressures during the first stage of normal labour. Am Amniotic fluid pressure EL head to cervix pressure at the equator of the fetal head -6 head to cervix pressure 6 cm below the equator of the fetal head. The head to cervix pressure at the equator increases during the progress of the first stage because of the increased amniotic fluid pressure.



P 33
2



Fig 2 Mouldings of the skull bones in early first stage of normal labour *S* Symphysis *P* promontorium *PR* and

PL parietal bones *F* frontal bone *O* occipital bone. Note the mouldings at the arrows



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Fig 3 Mouldings of the skull bones in late first stage of normal labour *S* Symphysis *P* promontorium *PR* and

PL parietal bones *F* frontal bone *O* occipital bone. Moulding has increased. Same patient as in Fig 2.

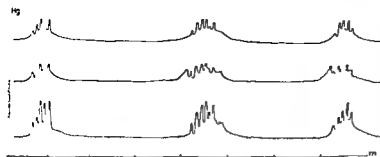


Fig 4 Recordings by using intra uterine tokometry in second stage of labour Am Amniotic fluid pressure E1 head to cervix pressure at the equator of the fetal head -3 Head to cervix pressure 3 cm below the equator of the fetal head Note the higher pressure in the level -3 corresponding to the distance of the pelvic floor

the corresponding amniotic fluid pressure. The various recorded head to cervix pressure decreases as the lower pool of the fetal head. During the first stage of labour the head to cervix pressure at the equator of the fetal head is on average the same as the same fluid pressure throughout the first stage except at the time of rupture of the membranes when the head to cervix pressure at the equator increases and the pressures at lower levels decrease. As the amniotic fluid pressure increases during the progress of the first stage of labour the head to cervix pressure at the equator of the fetal head increases (Fig 1).

The high head to cervix pressure at the equator causes a moulding of the skull bones (Fig 2). The parietal bones are elevated in relation to the frontal and occipital bones giving a level difference in the coronal and lambdoid sutures. This moulding increases during the progress of labour (Fig 3) corresponding to the increased head to cervix pressure at the equator of the fetal head and the prolonged time of the prevailing increased pressures.

During the second stage of labour the head to cervix pressure at the equator decreases (Fig 4) and the moulding of the head likewise (Fig 5). The head to cervix pressure at lower levels increases because of the high

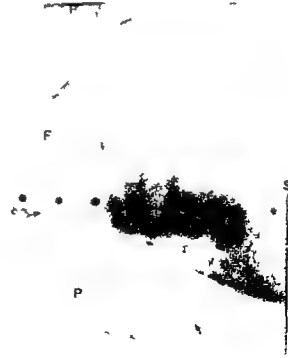


Fig 5 Fetal head during the second stage of normal labour S Symphysis P promontorium PR and PL

parietal bones F frontal bone O occipital bone Note the remoulding of the skull bones

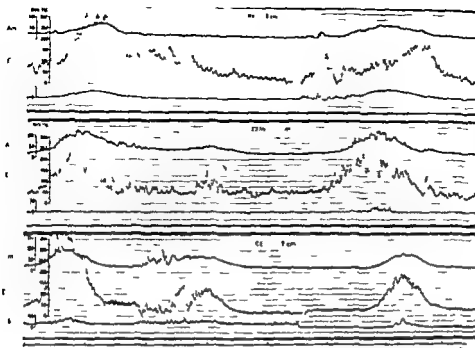


Fig 6 Recordings of the intra uterine pressures in spastic uterus. Am Amniotic fluid pressure. E head to cervix pressure at the equator of the fetal head. C head to cervix pressure 6 cm below the equator of the fetal head.

Note the high head to cervix pressure at the C caused by spastic contractions appearing as a saw-tooth pattern added to the pressure curves.



Fig 7 Mouldings of the skull born in spastic uterus. P Parietal bone. F frontal bone. O occipital bone. Note the large moulding between the occipital and parietal bones.

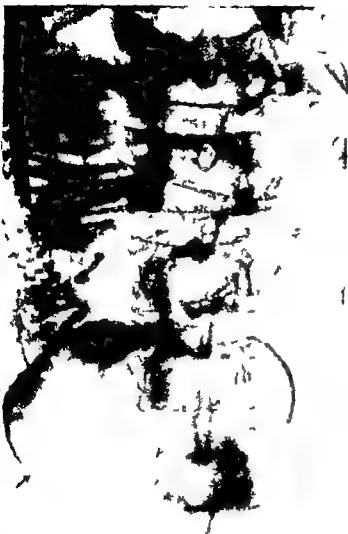


Fig 8 Mouldings of the skull bones in breech presentation. Note the impression of the parietal bone at the arrow (1)

distance of the pelvic floor. The latter may cause rupture of the perineum especially in primigravidae but the risk is reduced by pudendal block anaesthesia and episiotomy.

normal uterine action

In some cases of hypertonic inertia, spastic contractions occur in the annular musculature of the lower part of the uterus (Fig 6). The head to cervix pressure increases and moulding likewise (Fig 7). The moulding is otherwise the same type as in normal labour. The dislocation of the skull bones can be large—up to 25 mm in this type of peritonic inertia. In a material of 56 such patients all delivered and selected among women with violent contractions 16 or (29%) of the infants died all due to rupture of the tentorium. In such cases caesarean section ought to be done as soon as the diagnosis has been verified.

Cervical rigidity may cause an increased head to cervix pressure but can be successfully treated by the vibration method.

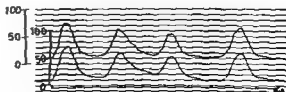


Fig 9 Amniotic fluid pressure recorded by two strain gauges in breech presentation. The upper curve shows the measurement in the middle part of the uterine cavity the lower curve the pressure between the fundus and the fetal head.

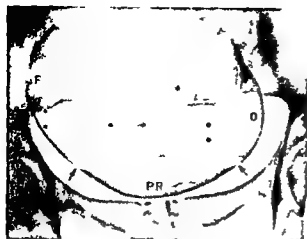


Fig 10 Contracted pelvis of the inlet *S* Symphysis *P* promontorium *PR* and *PL* parietal bones *F* frontal



bone *O* occipital bone Note the moulding between the parietal bones

Breech presentation

The risk of rupture of the tentorium of the fetus at vaginal delivery is rather high in breech presentation—higher than in vertex presentation. In the material referred above rupture of the tentorium was seen in 27% of the infants who died perinatally in comparison with 15% in vertex presentation.

During the first stage the pressure of the amniotic fluid on the fetal head is less than that of the head on the cervix in vertex presentation and likewise also the moulding (Fig 8). The pressure is the same on the various skull bones

(Fig 9) but their resistance to the increased amniotic pressure differs during contractions which explains moulding. The risk of rupture of the tentorium is small.

During the second stage the fetal head passes the birth canal quite rapidly and with the fronto-occipital circumference perpendicular to the direction of fetal movement but on the contrary in breech presentation the oblique circumference in vertex presentation. The larger the pelvis the wider must the birth canal be to avoid injury to the fetal head.

However the increased risk of lesions to the fetal head

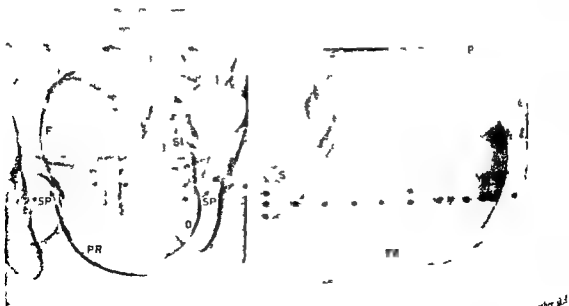


Fig 11 Contracted pelvis of the outlet *S* Symphysis *P* promontorium *SP* spines *PR* and *PL* parietal bones *F* frontal bone *O* occipital bone Note the moulding between the parietal bones but also between other skull bones

tween the parietal bones but also between other skull bones

each presentation is not only explained by the increased risk of contracted pelvis. The cervical dilatation is controlled by two main factors—the wedge effect of the leading part of the fetus and the brachystasis of the muscle cells of the corpus. The circumference of the rump is smaller than that of the fetal head and still smaller when vertex are presented. When the cervix is dilated for the same time the wedge effect is no longer acting. The cervical dilatation depends on the mechanism of brachystasis of the muscle cells of the corpus *only* when it takes longer time. Extraction of the fetus in this case of labour increases the risk of rupture of the membranes and it is important to adopt a wait and see policy.

Contracted pelvis

In contracted pelvis special mouldings are seen (Fig. 10) as compared with the moulding caused by narrow inlet. In normal labour the moulding caused by the cervix elevates both the parietal bones in the same degree, but in contracted pelvis one bone is elevated, giving a level difference in the fetal suture. Fig. 11 shows the level difference between the parietal bones but also between other skull bones in a case of narrow outlet, which also are seen in narrow inlet. When one tries to deliver the patient by vacuum extraction the moulding increases and the risk of brain lesions of the fetus increases. By using forceps the risk of brain lesions of the fetus is less, but the delayed operation may increase the risk of fetal distress and the risk of lesions of the membranes increases. Therefore the method to choose is the caesarean section in cases of contracted pelvis.

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LATE SEQUELAE OF INDUCED ABORTION IN PRIMIGRAVIDAE

The Outcome of the Subsequent Pregnancies

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Abstract In a retrospective study a group of 137 women legal abortion in their first pregnancy was compared a group of 133 women with spontaneous abortion (8 weeks) and a group of 19 women who had a (beyond 18 weeks) in their first pregnancy. Three of ectopic pregnancy were recorded in the second pregnancy in the legal abortion group, none in the other groups. The delivery group showed the best reproductive performance while the spontaneous abortion group had highest frequency of early abortion and the induced abortion group the highest frequency of late spontaneous abortion and premature delivery and in addition a trend towards earlier spontaneous onset of labour in their second pregnancy. There was a conspicuous decline of the reproductive performance in the third and fourth pregnancy of the induced abortion group and a highly significant increase of the rate of late abortion compared to the overall rate of late abortion in the department. The induced abortion group showed an increased rate of spontaneous primary and premature rupture of the membranes and also a definite trend towards lower weight of newborn, especially beyond 41 weeks of pregnancy. There was a close correlation between induced abortion in a woman below 17 years of age on the one hand and repeated abortion and/or unmarried state in the second pregnancy on the other. A significant correlation was revealed between early menarche and legal abortion in the first pregnancy and also between early menarche and unmarried state in the first pregnancy as found in a random series of first pregnant women.

Early complications of induced abortion are thoroughly analysed in the medical literature but there are relatively few publications dealing with the more important aspect of late sequelae. This is a regrettable situation, especially because a large and increasing number of young women have their first pregnancy terminated by induced abortion. Harmful effects on later reproductive performance are of great importance for this category of women than for those who already have a family.

One of the main reasons for the conspicuous scarcity of follow up reports on subsequent pregnancies in women who have had their first pregnancy terminate probably is the risk that a prospective follow up procedure might be emotionally very disturbing and create a great deal of unhappiness among the women concerned.

The present study is retrospective and based on hospital records. In some areas incomplete information had to be accepted in order to maintain the strictest secrecy.

MATERIAL AND METHODS

The report is based on the record of the obstetrical department of a combined regional and university clinic where admittance is accepted for geographical as well as medical reasons. For this reason the results in the study group cannot be compared with the general data from the Norwegian Medical Birth Registry.

Statistically reliable groups must be provided from the clinic in question. A study group and 2 control groups were established. The number of cases in the groups is not the same because cases with multiple pregnancy and non-obstetrical diseases were excluded after collection of the material.

Group 1 comprises 137 women, the total number of women admitted as pregnant from 1972 to November 15th 1974, who according to their records had had legal abortion of their first pregnancy. Of the 137 induced abortions of the first pregnancy 96 were performed at our department, 31 at other Norwegian hospitals and 10 abroad (U.K.). Detail of the operation in these last 41 cases were missing, but there were no indications of major complications. The methods of abortion in our department in the presented cases were M & C vacuum aspiration and intra-amniotic hypertonic saline. It appears that a curettage was always used as a final step of a suction procedure in order to make sure that no products were retained. Dilatation of the cervix in one step was only performed below 10 weeks of pregnancy. In more advanced pregnancies gradual dilata-

Table I

	Group I	Group II	Group III
Age at first pregnancy	19.5 ± 3.4 (15-33)	23.4 ± 3.9 (16-33)	23.7 ± 3.4 (15-33)
Age at second pregnancy	22.2 ± 4.2 (16-40)	25.7 ± 4.1 (17-35)	6.7 ± 3.8 (15-33)
Not married at second pregnancy	36/137 (26.3%)	5/133 (3.8%)	2/129 (1.6%)
Number of cases where the age at menarche is known	129/137 (94.2%)	178/133 (96.2%)	125/129 (96.9%)
Number of cases with early menarche (before 13 years of age)	41/129 (31.8%)	50/178 (28.1%)	41/125 (32.8%)

tation was achieved over 12-14 hours with Lovset's dilatator—an instrument with two steel branches gently opened by continuous traction by a metal string in such a way that the cervical opening is never blocked as with the use of laminaria.

Group II comprises 133 women collected at random from the same period with a record of spontaneous abortion before 28 weeks in their first pregnancy.

Group III comprises 129 women collected at random from the same period with a record of delivery after 28 weeks or more in their first pregnancy.

The statistical methods have been Yates' corrected Chi-square, Fisher's exact probability tests and Wilcoxon's test for two samples (two-tailed).

RESULTS

The characteristics of the groups and the outcome of the second pregnancy are compared. Then the outcome of the third and fourth pregnancy in the study group is presented and some trends compared with those of the total material of the department.

Data on age, married state and menarche are given in Table I. The mean age of the women in the study group was 4 years below that of the other groups. This is undoubtedly related to the fact that

young age represents one of the indications for legal abortion.

There is a significantly ($p < 0.001$) higher percentage of women in the study group who were unmarried at their second pregnancy compared with each of the other groups. The findings indicate a relatively low degree of social adjustment as far as the married state is concerned in the women who had their first pregnancy terminated by legal abortion. This is especially true for those who have had their pregnancies terminated before they were 20 years of age. They comprise a far greater part of the unmarried than the married women in the study group ($p = 0.003$).

Relatively early sexual maturation indicated by menarche before 13 years of age was more frequently found in the study group than in group II ($p = 0.03$). Group II had the highest frequency but not significantly different from the study group. This means that both groups of women who had suffered abortion in their first pregnancy had had relatively early sexual maturation.

That early menarche predisposes to an unwanted pregnancy is rather self-evident. An unwanted preg-

Table II

Number with primary spontaneous rupture of the membranes within parentheses

Outcome of the second pregnancy	Groups			Total number
	I	II	III	
Legal abortion	7	0	6	13
Ectopic pregnancy	3	0	0	3
Early spontaneous abortions (below 14 weeks)	6	16	7	29
Late spontaneous abortions (14-21 weeks)	2 (1)	0	0	2
Premature delivery (birth weight 500-2499 g)	10 ^a (8)	7 (1)	3 (0)	20
Birth weight 2500 or more	109	110	118	337
Total number	137	133	129	399

^a 1 case where the relation between rupture of the membranes and onset of contractions are unknown.

^b 1 induced case
3 induced cases

Table III Interval in hours between spontaneous rupture of the membranes and onset of the contractions

Values	Groups			Total number
	I	II	III	
Less than 10 hours	10	13	19	42
10-20 hours	10	6	3	19
More than 20 hours	9	3	0	12
Total number	29	2	22	73
Total number of cases studied	97	90	107	289

may be terminated as in the study group may also be allowed to continue and then often an unmarried mother. In a random series of first pregnancies in our obstetric department menarche was found in 20 of 45 unmarried women i.e. 44.4% against 57 of 205 married women 27.8% a difference which is statistically significant ($p=0.03$).

The outcome of the second pregnancy in the 3 groups is shown in Table II.

Even of the women in the study group had also a second pregnancy terminated by legal abortion.

Five of these women were 16 years or younger at their first abortion. Even if ectopic pregnancy and legal abortion are excluded there was a significantly higher proportion ($p=0.007$) of very young women among those who have legal abortion in their first pregnancy than among those who accepted their second pregnancy.

Because the study group only comprises women who had become pregnant again nothing can be said about the infertility rate after legal abortion.

The series however contains one case who had had an induced abortion which was uncomplicated except for fever without local symptoms on the first postoperative day. She married 3 years later and proved to be infertile because of bilateral tubal occlusion. A plastic operation was performed on the tubes and five years later she had an ectopic pregnancy. In this case the induced abortion was probably the primary cause of the events leading to this complication. Ectopic pregnancy occurred otherwise twice in the study group 2 and 3 years respectively after the induced abortion. One of these abortions was also complicated by fever without local symptoms on the first postoperative day. This indicates that fever without local symptoms might be of more importance than generally considered.

The overall rate of ectopic pregnancy in the first pregnancy at the department for the period 1967-71 was 41/8765 i.e. 0.47%. If all 3 cases are accepted as a result of induced abortion the difference is significant ($p=0.03$).

Early spontaneous abortion (before 14 completed weeks of pregnancy) occurred significantly more frequently in group II where the women already had had a spontaneous abortion than in the other groups compared with the study group and the delivery group $p=0.03$ and 0.001 respectively.

Late spontaneous abortion (from 14 to 21 completed weeks or fetal weight of less than 500 g) occurred only in the study group. In group II however one case had been treated prophylactically with cervical suture because of suspected cervical incompetence and labour started immediately after the removal of the suture at term. In this case late abortion or premature delivery might have occurred if the woman had not been treated.

Table IV The outcome of the second, third and fourth pregnancy after induced abortion in the first pregnancy

Pregnancy	Percent in parentheses						Total number
	Ectopic	Induced abortion	Early abortion	Late abortion	Premature delivery	Mature delivery	
First pregnancy	3 (7.7)	7 (5.1)	6 (4.4)	2 (1.5)	10 (7.3)	109 (79.6)	137 (100.0)
Second pregnancy	0	5 (10.0)	4 (8.0)	5 (10.0)	5 (10.0)	31 (67.0)	50 (100.0)
Third pregnancy	0	1 (6.3)	2 (12.5)	2 (12.5)	1 (6.3)	9 (56.3)	15 (100.0)
Fourth pregnancy	3 (1.5)	13 (6.4)	13 (6.4)	9 (4.4)	17 (8.4)	149 (73.4)	203 (100.0)

Birth weight 500-999 g

Table V *Consecutive induced abortions*

First pregnancy					Second pregnancy				
Age	Married	Weeks of pregnancy	Complications		Interval (months)	Age	Married	Weeks of pregnancy	Complications
1	16	No	7-8	None	5	17	No	12	None
2	15	No	9	?	9	16	No	9	?
3	19	No	7-8	None	7	20	No	9	?
4	16	No	22	None	12	18	No	12	None
5	16	No	11-12	None	11	17	No	10-11	None
6	20	No	11	None	5	21	No	8-10	None
7	16	No	11	Bleeding Repeated curettage	5	17	No	9	?

Premature delivery (birth weight 500-2499 g) Only in the study group was there 1 case between 500 and 1000 g. One case in the study group and 3 cases in group II were induced because of severe preeclampsia. If these cases in addition to the cases with induced abortion and ectopic pregnancy are excluded there is a significantly higher rate of late spontaneous abortion and premature delivery combined in the study group than in the other groups combined ($p=0.002$).

Duration of pregnancy more than 40 weeks was significantly less frequent in the study group where 20% the induction rate was lower than in the two other groups combined ($p=0.006$). This indicates a trend towards earlier spontaneous onset of labour.

If a cervical lesion is the factor responsible for this trend and for the increased frequency of late spontaneous abortion and premature delivery one would expect a higher rate of primary as well as premature rupture of the membranes (Table III).

Primary rupture of the membranes defined as an interval of 6 hours or more between this event and onset of labour pains was more frequent in the study group than in each of the other groups. Compared with group III the difference was highly significant ($p<0.001$) but did not reach the level of significance for group II ($p<0.10$). Late abortion and premature delivery started more often with primary spontaneous rupture of the mem-

the study group than in the other groups combined ($p=0.01$). See Table II.

Incompetent cervix however may not be the only responsible factor. This was indicated by a case in the study group who after a late spontaneous abortion in the second pregnancy was treated prophylactically with a cervical suture in the third pregnancy. The outcome was nevertheless a premature baby.

The duration of labour after starting of contractions was very similar in the groups of women who had had an abortion in their first pregnancy. The women who had passed 28 weeks of pregnancy however had a highly significant shorter duration of labour ($p<0.001$).

The weight of the newborn after 36 weeks of pregnancy was significantly lower ($p<0.001$) in the study group than in the other groups and did not even rarely reach 3500 g ($p<0.001$). The weight was analysed separately for each week of pregnancy and it turned out that except for 36 weeks the difference was mainly caused by a significant difference after 41 weeks of pregnancy. Group I/group II $p<0.005$ and group I/group III $p<0.01$. The findings seem to be due to intrauterine growth limitation than an early starting of labour. Intrauterine adhesions or other pathological processes limiting the capacity of the uterus occur after an induced abortion.

Pregnancy	Outcome of previous pregnancy	Fourth pregnancy		
		Age	Married	Outcome of pregnancy
1st	Late spontaneous abortion 16 weeks	22	Yes	Stillbirth 40 weeks 1450 g
2nd	Late abortion 18 weeks			
3rd	Full term 3740 g			
4th	Full term 3180 g			
5th	Full term 3160 g			
6th	Full term 3330 g			
7th	Criminal abortion Bleeding Infection	21	Yes	Full term 3480 g

10 cases of immunization one of Rh and one of type occurred in the study group none in the other groups. The perinatal mortality was highest in the late spontaneous abortion group but there was no significant difference between the 3 groups. Only 1 of the prematurely delivered babies in the study group died but because many of them had a very low birth weight later sequelae may occur. The outcome of the total number of pregnancies recorded after induced abortion of the first pregnancy is given in Table IV. Repeated induced abortions were of two types: consecutive and intermittent with one or two intervening pregnancies. A survey of the consecutive induced abortions is given in Table V. Five of the seven cases were 16 years or younger at their first abortion as stated above. Cases 1 and 2 both suffered late spontaneous abortion in the third pregnancy while the fourth pregnancy in case 1 had an particularly unhappy outcome: a stillborn small dated fetus. She was a highly nervous type and was treated with sedatives. Case 7 illustrates that even legal abortions and one complicated criminal abortion are not incompatible with a happy outcome of a fourth pregnancy. In addition there are 6 induced abortions of the intermittent type. They had had their first abortion

from 16 to 20 years of age: mean age 17.8 ± 1.5 years at an earlier age than the average in the study group.

These findings indicate that women with induced abortions at an early age represent a high risk group for unwanted pregnancies. Both social problems and especial vulnerability of young girls to the mental trauma of induced abortion may be responsible for this.

Table IV shows a gradual rising rate of both early and late spontaneous abortion as well as premature delivery from the second to the fourth pregnancy.

The cases with late spontaneous abortion (14-21 weeks of pregnancy) were all treated at our department and the frequency in the study group consequently can be compared with the overall frequency. Table VI shows a highly significant increased rate of late abortion in the study group. The table also shows a gradual rising of the rate in the total material. A rising rate of late abortion is therefore not a specific trend for the study group as shown in Table IV. Both increasing age and trauma of the reproductive tract may be responsible.

Characteristics in the study group with possible relation to late abortion and premature delivery

The age of the woman at the abortion. More of the women who suffered late abortion or premature delivery had had their first pregnancy terminated before the age of 18 than those with full term delivery (birthweight 2500 g or more). The difference however was not statistically significant ($0.1 > p > 0.005$).

Duration of pregnancy at the time of abortion. was unknown in 41 cases. Compared to the general distribution of duration in the 96 abortions performed in our department the patients who subsequently suffered late abortion or premature delivery had a more favourable distribution with nearly doubled frequency of early pregnancies of 11 weeks duration or less.

Early complication rate in the study group was 13.5% and in those of the group who later suffered late abortion or premature delivery 2/18 i.e. 11.1%. Uncomplicated induced abortion represents evidently no guarantee against complications in a later pregnancy.

The details of the abortion techniques were unknown in as many as 41 cases. The material is therefore poorly suitable for an analysis of the significance of the abortion procedure. We did

Table VI Late abortion (14-21 completed weeks)

Number and rate as to number of pregnancies (induced abortions early abortions and ectopic pregnancies excluded) Per cent in parentheses

Group I	Over all occurrence at the department 1967-1971	Statistical difference
2/121 (1.65) Second pregnancy	5/8765 (0.057) Recorded as first pregnancy	$p < 0.002$
5/41 (12.20) Third pregnancy	9/5592 (0.161) Recorded as second pregnancy	$p < 0.001$
4/13 (30.77) Fourth pregnancy	13/5977 (0.218) Recorded as third pregnancy	$p < 0.001$

Number of cases with previous induced abortion ectopic pregnancy and early abortion are not recorded

however not expect to find a tendency to an inverse correlation between the duration of the pregnancy at the time of abortion and also the rate of early complication on one hand and later sequelae in the subsequent pregnancies on the other. The operation procedures were therefore more closely examined especially the degree of dilatation of the cervix. It was found that this had exceeded 10 mm in the large majority of cases. Even if the abortions appeared to be uncomplicated the cervical canal may have been damaged.

Two of the three women in the study group whose second pregnancy was extrauterine had fever without local symptoms on the first postoperative day. This type of complication may be more important than is generally considered.

DISCUSSION

An association between induced abortion and ectopic pregnancy has been suggested in several reports and confirmed in a case control approach (6). The finding of 3 cases of ectopic pregnancy in the legal abortion group fits with this hypothesis. Considering the unquestionable risk of infection connected with induced abortion no wonder that an ectopic pregnancy occasionally may be caused by this operation. Two of the three patients had fever without local symptoms on the first postoperative day.

The results confirm previous publications reporting of an increased rate of late abortion and premature delivery (5, 7, 8) and also a trend towards

earlier onset of labour (5, 7) in the second pregnancy after legal abortion of the first.

It is accepted as a general trend in reproductive performance that the prognosis is better for the second delivery than the first and then gets poorer with increasing number of deliveries. The general trend does not hold for the women who were legally terminated in their first pregnancy. As shown in Table IV there is a conspicuous decline in the reproductive performance in the third and fourth pregnancy, a long time effect that as far as we know has not been demonstrated before. The findings of a significantly increased and more frequent rate of late abortion indicate that the termination of the first pregnancy had represented a major trauma.

The trend towards earlier spontaneous onset of labour, premature delivery and late abortion may be caused by similar mechanism or mechanisms. Several observations indicate that cervical incompetence may play a part in the pathogenesis. Increased rate of primary and premature rupture of the membranes has been demonstrated in the present study and also by Lembrich (5). This strengthens the hypothesis of a cervical lesion. It has however been observed that cervical incompetence does not always prevent early onset of labour.

In the present study the weight of the newborn in the study group proved to be relatively low compared with the control groups and especially for pregnancies of more than 41 weeks duration. In addition there is an example of severe growth retardation in the fourth pregnancy where the woman had had two consecutive abortions (Table I). These findings seem to indicate that not only a cervical but also a corporal lesion may contribute to the reproductive failures. Induced abortion is a type of operation that may predispose to intrauterine adhesions (4). The possibility of a partial thrombosis of the uterine vessels caused by the sudden interruption of a rapidly developing uteroplacental circulation must also be kept in mind.

One of the girls with repeated abortions who had a particularly unhappy reproductive history appeared after the first abortion. Bahr and Bjerkedahl (1) found that women with repeated experience reproductive complications and failures. A multifactorial etiology in reproductive failure is probably often at play.

In this relatively small material the increased rate of late reproductive sequelae in the very first

its did not reach statistical significance. The however has revealed a close correlation between induced abortion in girls below 17 years of age on the one hand and repeated induced abortion and as unmarried state in the second pregnancy on the other. These girls comprise undoubtedly a risk group for unwanted pregnancies.

Results from the present study strongly indicate that induced abortion in primigravidae especially the very young ones is far from an innocuous procedure. It remains to be proved that new techniques can make it safer. The psychological trauma is a rate inevitable and prophylactic measures to reduce the frequency of unwanted pregnancies especially at an early age are highly desirable.

The present study has revealed a significant correlation between early menarche on the one hand and induced abortion as well as unmarried state in first pregnancy on the other. A significant correlation between pregnancy before 15 years of age and early menarche was demonstrated by Duenhoelter et al. (2). That early sexual maturation increases the risk of unwanted pregnancy is also a self evident and girls with early menarche should be considered a high risk group in this respect.

A explanation can be offered for the finding that early menarche also was correlated with spontaneous abortion in the first pregnancy.

There was a non significant trend towards negative correlation between duration of the pregnancy and time of termination as well as complication

rate on the one hand and late abortion or premature delivery on the other. Cervical dilatation of more than 10 mm may be responsible for this (3).

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MATERNAL DEATHS IN ICELAND 1911-1975

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DEFINITION

This report on maternal deaths in Iceland are based on all women who died after the 28th week of pregnancy and during the first eight weeks after delivery are included irrespective of the cause of death. Out of 225 891 women who delivered during the 65 year period 371 women died. Maternal deaths per thousand deliveries declined from 3.89 to 0.09 from the first 5 year period (1911-1915) to the last 5 years of the observation time (1971-1975). The three main causes of maternal deaths: puerperal fever, haemorrhage and toxæmia are discussed. Since the new Maternity Clinic at Landspítalinn was opened in 1949 it has been the main department for obstetrics and gynaecology in Iceland. In which a great majority of complicated cases in pregnancy and delivery are treated. Since 1949 39 411 women have delivered at this institution. There have been 15 maternal deaths at the institution or 0.4 per thousand. A separate table (Table III) shows the age, parity and causes of death in the 15 cases of maternal deaths in Iceland during the last decade occurring per 11 000 deliveries. This is a problem of communication as well as a medical problem.

INTRODUCTION

Law requiring a death certificate for all deaths was passed in Iceland in 1911. Consequently quite accurate sources concerning the various causes of deaths in Iceland are available since that year. The Statistical Bureau of Iceland has collected all death certificates since 1911 and publishes data concerning the various causes of death at 5 to 10 year intervals (1).

In a recent report on deliveries in Iceland in the past (2) various aspects of maternity services was discussed and maternal deaths since 1881 summarized.

As mentioned in the report there have been remarkable changes in the maternity services in Iceland particularly during the last three decades.

Landspítalinn—the University Hospital—was established in 1930. It included a small maternity unit of 14 beds, in fact the first maternity department in the country.

Originally this unit was supposed to take care of complicated cases only. Prior to this time practically all women in the country had delivered at home.

During the following two decades a number of maternity homes and rural hospitals were founded. The number of deliveries at these institutions increased steadily as the years passed.

In 1949 a new department of obstetrics and gynaecology opened at Landspítalinn with approximately 60 beds. This department has since its opening accounted for approximately 40% of all deliveries in Iceland and the majority of all complicated deliveries.

Presently 99% of all women in Iceland deliver in hospitals and maternity homes and over 70% in hospitals supervised by specialists in obstetrics and pediatrics.

MATERNAL DEATHS SINCE 1911

Table I shows the main causes of maternal deaths in the country 1911-1975, the total number of deliveries and death rate per 1 000 deliveries at 5 year intervals.

In spite of the small number of cases Table I shows clearly that the same evolution has taken place in Iceland as in other countries.

Table I *Maternal deaths in Iceland 1911-1975*

	Causes of maternal death				Total	Total number of deliveries	Maternal deaths per 1 000 deliveries
	Puerperal fever	Haemorrhage	Eclampsia toxæmia	Other causes			
1911-1915	19	7	14	6	46	11 806	3.89
1916-1920	15	1	8	12	36	12 579	2.85
1921-1925	23	3	9	5	40	13 162	3.09
1926-1930	13	13	8	3	37	13 661	2.71
1931-1935	12	14	3	7	36	13 461	2.67
1936-1940	10	7	4	7	28	12 430	2.25
1941-1945	13	8	10	8	39	15 818	2.46
1946-1950	4	6	7	4	21	19 778	1.08
1951-1955	1	1	6	5	13	21 451	0.61
1956-1960	0	5	5	1	11	24 179	0.46
1961-1965	1	3	4	2	10	23 931	0.41
1966-1970	0	1	1	0	2	21 811	0.09
1971-1975	0	1	1	0	2	22 374	0.09
Total	111	70	80	60	321	275 891	1.47

Puerperal fever was a rather common cause of maternal death until the end of the second world war when chemotherapeutics and antibiotics came into common use. During the last 25 years there have only been two maternal deaths caused by puerperal fever registered.

Haemorrhage before, during or after delivery has been a rather common cause of maternal death. Better facilities and therapy in the birth institutions (shock therapy, blood transfusions etc.) during the last three decades explain the lowering of maternal death in this group.

The third main group of maternal death—eclampsia—toxaemia has declined remarkably during the last 25 years as well as the fourth group—other causes. As mentioned earlier, the main reason for the decline in maternal death by the above named causes is a better antenatal care in the country as well as better communication between the local health personnel and the major birth institutions.

Under the heading 'other causes' are listed various diagnoses such as pulmonary embolism, apoplexy, heart diseases, cancer etc. Finally Table I shows that the maternal deaths in Iceland have decreased from 3.89 per 1 000 at the beginning of the observation period to 0.09 per 1 000 deliveries at the present time.

Table II *Number of deliveries and maternal deaths at the Landspítalinn Maternity Clinic 1949-1975*

Years	Number of deliveries	Maternal deaths	Maternal deaths per 1 000
1949-1960	19 247	11	0.6
1961-1975	20 164	4	0.2
Total	39 411	15	0.4

MATERNAL DEATHS AT THE LANDSPÍTALINN MATERNITY CLINIC

As mentioned earlier, the new Maternity Clinic at Landspítalinn opened in 1949. During the next decade complicated cases of pregnancy and delivery were admitted to the new department from various parts of the country on an ever increasing scale. During this period communications by land and particularly by air made the transport

Table III *Causes of maternal deaths at the Landspítalinn Maternity Clinic 1949-1975*

No	Year	Age	Parity	Causes of death
1	1950	25	IV	Haemorrhage post partum placenta
2	1950	31	0	Haemorrhage post partum uteri
3	1952	29	III	Ruptura uteri
4	1953	35	0	Eclampsia
5	1954	25	I	Toxæmia matris. Haemorrhage et embolia uteri
6	1954	25	II	Embolia pulmonum post partum
7	1955	34	II	Caesarea
8	1955	24	I	Eclampsia
9	1956	33	II	Incompetent cervix uteri
10	1956	47	I	cordis congen
11	1959	44	V	Ruptura uteri
12	1961	23	0	Preeclampsia. AMU placenta
13	1965	41	VII	Apoplexia cerebri
14	1971	27	0	Cancer ovarii
15	1971	30	II	Embolia pulmonum
				Eclampsia
				Haemorrhage post partum cervicis uteri

All diagnoses confirmed by autopsy

is within the country easier even from the most remote areas

21 maternal deaths during the years 1946-1950 only occurred in the Landspítalinn Maternity Clinic of 34 women who died during the 15 year period 1965 nine died at the Maternity Clinic but from the who died during the last 10 years two died at the and in fact both during the latter half of the period during the last decade practically all high risk cases been admitted to institutions offering the best facilities

Table II shows the number of deliveries and maternal deaths in the Landspítalinn Maternity Clinic since 1949 the first 11 years are compared with the last 15 years the number of women in each group is comparable Table III shows the causes of maternal deaths at the Landspítalinn Maternity Clinic since 1949 a further analysis of the causes of death shows that six died from haemorrhage caused by rupture of the uterus in three cases Four cases were moribund on arrival

one died from eclampsia three shortly after arrival at the department Two died suddenly from pulmonary embolism several days after delivery Both of them had an extensive thrombo-phlebitis in the femoral and iliac veins as well as leaves three patients who died from causes not related to pregnancy or its complications

Congenital heart disease
Cerebral apoplexy where postmortem examination revealed aneurysm of the cerebral arteries
Cancer of the ovary discovered by an explorative laparotomy when the patient was 4 months pregnant She had widespread metastases and ascitis already at that time

An attempt was made to save the child by Caesarean section in the eighth month of pregnancy but the baby died from hyaline membrane disease on the second day the mother succumbed to her disease 7 weeks after the delivery

DISCUSSION

This material shows that maternal deaths in Iceland are a rare problem at the present time During

the last 10 years four women out of the 44 000 who delivered died or one per 11 000 deliveries

Iceland is a sparsely populated country of 103 000 square kilometers (over 40 000 square miles) with a population (1976) of 220 000

Seventy to eighty per cent of the population lives within reasonable distance from hospitals and delivery homes However for several years to come approximately one fourth of the population will continue to live far away from hospitals and in a few instances in very remote areas

Thus we are well aware of the fact that some parts of the country are denied modern hospital facilities during the winter season and accidents will happen away from hospitals birth catastrophes as well as other acute diseases or traumata

In conclusion maternal deaths in Iceland are today a problem of communications as well as a medical problem

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Submitted for Festschrift

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rekommenderar
det lagst doserade tillförlitliga p-pillret i varje situation

Alla nya P-piller-
patienter



Neovletta

Kvinnor som måste
ställas om p.g.a.
biverkningar med
andra P-piller



Neovletta

Kvinnor som måste
ställas om p.g.a. svåra
blödningsproblem
med andra P-piller



Regunon

NEOVLETTA - 150 ug \bar{n} norgestrel 30 ug etinylostradiol

REGUNON - 125 ug \bar{n} norgestrel 50 ug etinylostradiol



Tillverkare Schering AG
Ombud Schering Nordiska AB
Fack 131 01 Nacka

MATERNAL MORTALITY IN AUSTRALIA 1964-72

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Statistics covering deaths directly due to pregnancy in the Commonwealth of Australia have been available since the turn of the century. However those for deaths associated with pregnancy have not. Over the past decades each State in the Commonwealth has set up Maternal Mortality Committees to collect confidential information on each maternal death, both direct (where the death is directly attributable to pregnancy or childbirth) and indirect (where the death is associated with but not directly due to pregnancy or childbirth). New South Wales set up the first such Committee in the 1930's and this has since been followed by the other States. Most of the reports have been published from time to time but owing to the small numbers involved these reports have been limited in the conclusions that could be made. Confidential information on each maternal death is obtained and is considered by the State Maternal Mortality Committee. The cause of death is confirmed and classified. A post-mortem is usually performed in each instance, the pathologist travelling to the town where the death has occurred if requested.

When all the information is collected the case history is assessed and a decision made as to whether there has been a primary avoidable factor present. Such a factor is judged to be present if there has been some departure from accepted standards of satisfactory care which may have played a part in the ensuing death. It is not suggested that in all cases in which avoidable factors are considered present death could certainly have been prevented, but the presence of an avoidable factor is regarded as an indication that the risk of death could have been at least materially lessened. It is decided that there is an avoidable factor present further classification is made as to whether the medical attendant, the hospital or the patient is been responsible for the departure from accepted practice. Due cognizance is taken of the geographical area where the death has occurred, the facilities that have been available and the status of the medical attendant.

The individual State and Territory statistics have been collected and published compositely on a triennial basis, the first one being for 1964-66 followed by reports for the years 1967-69 and 1970-72.

The purpose of the reports is one of education to inform the medical and nursing profession of the major causes of death and to highlight the avoidable factors found and so improve the obstetric service of the country.

There were 275 maternal deaths in the first triennium (1964-66), 237 in the second (1967-69) and 244 in the third (1970-72)—a total of 756.

Table I gives the approximated maternal mortality rates for each triennium. It is noted that there has been a significant reduction in the deaths directly due to pregnancy in the last triennium whereas there has been no change in the number of associated deaths in the three triennia. An attempt to explain the reason for this reduction will be made later in this paper. A comparison between the deaths in Australia directly attributed to pregnancy—18.96 per 10 000 live births in 1970-72—to other countries reveals that Scandinavian countries vary between 10.8 and 18.0, England and Scotland approximately 20 and Canada and USA 26.

Table II shows the incidences of the five main causes of death over the three 3 year periods. Although the condition was present it may not have been the major cause of death. These conditions will be discussed individually.

Haemorrhage

Thus the largest group is made up of several different conditions, namely abruptio placentae (22), placenta praevia (13), ruptured uterus (35), ectopic pregnancy (19) and post partum haemorrhage (52). Assessment of the histories showed that there was an avoidable factor in over 66% of cases.

Table I Approximate maternal mortality rates

Triennium	Total no of confinements	Maternal deaths	
		Number	Rate per 10 ⁵ confinements
1964-66	667 649	202 (73) ^a	30.25 (10.93)
1967-69	713 064	166 (71)	23.27 (9.95)
1970-72	790 818	150 (94)	18.96 (11.88)
1964-72	2 171 531	518 (238)	23.85 (10.96)

Deaths directly attributable to pregnancy or childbirth
^a Deaths associated with pregnancy or childbirth but not directly due to pregnancy or childbirth

The importance of treating anaemia during pregnancy, the availability of blood and its prompt and adequate replacement cannot be stressed enough. Prevention of blood loss by prophylactic oxytocics and the careful repair of lacerations can avoid many haemorrhages. Routine grouping and estimation of haemoglobin levels in all patients is a necessity. It must not be forgotten that the number of deaths in which an avoidable factor was considered present is only the tip of the iceberg. There are many patients who almost die from haemorrhage be it ante-, intra- or post partum. The prevention of these near misses is also essential.

Some of these deaths occur in remote parts of the country where the aborigine who refuses medical aid during pregnancy and childbirth lives. Another problem is the storage of blood in remote areas. In rare instances a massive transfusion is required to save the life of the mother and the amount of blood available may be insufficient. The continued occurrence of death from ectopic pregnancy is due to either misdiagnosis on the part of the practitioner or to failure of the patient to call the doctor.

Deaths due to ante partum haemorrhage are not great but in each instance an avoidable factor is usually present.

Infection

Deaths from infection have remained remarkably constant over the nine year period. The infection is usually classified as genital or extra genital in origin. Included under the classification of genital sites are deaths due to septic abortion and those associated with vaginal delivery and Caesarean section.

The 41 deaths associated with septic abortion will be mentioned later. There were 15 deaths associated with Caesarean section. Bacteraemic

shock was not a common cause of death (11.2%). In the extra genital sites pulmonary infection was the commonest cause of death. Any infection in pregnancy must be regarded seriously, especially if there is a cardiac complication present or if an anaesthetic is required. Patients with pre-existing bronchiectasis or asthma must be under constant supervision during the last 6 months when viral infections are more common.

Most deaths from infection must be regarded as avoidable. If any impact is to be made on infection as a cause of death, a 24 hour adequate bacteriological service must be available so that the required antibiotic can be given. Prophylactic antibiotics are necessary in specific obstetric situations and bacteraemic shock must be thought of whenever a patient collapses immediately after delivery.

Pulmonary embolism

Deaths from pulmonary embolism are usually unexpected and very distressing for the relatives. Table II shows the number of deaths in each triennium and the times of their occurrences. There is a marked reduction in the number of deaths in the last triennium.

Deaths after vaginal delivery were the main factor in this reduction. In the first triennium deaths without warning far outnumbered those in which there was a warning. In the last triennium the numbers were about the same. Only half of the patients with warning signs had treatment. Deaths during pregnancy usually occurred in the elderly, multigravid patient with varicose veins who on examination was found to have a deep vein thrombosis. Deaths after Caesarean section are rare, especially since early ambulation and a low dose heparin regime have now become routine.

An interesting observation regarding the pro-

Table II Incidences of main causes of maternal mortality

Cause of death	Years			
	1964-66	1967-69	1970-72	Total
Haemorrhage	75	47	41	163
Infection	47	38	41	126
Pulmonary embolism	38	43	22	103
Abortion	45	5	37	87
Toxaemia	43	77	1	121

III Pulmonary thrombo-embolism

	Number of deaths		
	1964-66	1967-69	1970-72
during pregnancy	3	15	5
after vaginal delivery	22	23	10
after Caesarean section	13	5	4
after abortion	6	—	2
after operation for pre pregnancy	—	—	1
	44	43	22

in deaths is the time at which the embolism occurs. Approximately 25% occur within 24 hours of delivery and another 25% within the first week. Breakdown of patients dying from pulmonary embolism reveals that the risk increases with age parity. This may be the reason for the fall in the number of deaths for over the nine years studied. The number of elderly multigravidae has been markedly reduced. As nations come more to zero population growth deaths from pulmonary embolism become much less. Meantime the patient with greatest risk is the obese elderly multigravidae with severe varicosities of the lower extremities. Warning signs are more common in the patient who has an ante partum embolus. The use of anticoagulant has now become routine when deep vein thrombosis is diagnosed and after Caesarean section when low dose heparin is used.

Abortion
The following abortion continue to occur despite relaxation of indications for legal abortion in the country. The partial elimination of untrained abortionists has not markedly reduced the number of deaths which has always been low in any case. The following legal abortions are now occurring indicating that the procedure is not without risk. The two main causes of death following abortion are sepsis and haemorrhage, sepsis being the more common. However it must be noted that in the triennium less than 15 women died in Australia from septic abortion. In scrutinizing deaths from abortion it is noted that a proportion die from the condition for which abortion was performed. In others the technique of the medical attendant was faulty. In the vast

majority the pregnancy could have been prevented if the patient had availed herself of contraceptive advice which is available or had she immediately changed from one technique which was not suiting her to another. In theory because therapeutic abortions are carried out to save life all deaths in this group should be avoidable.

Toxaemia

Included in this group are patients who died from pre-eclampsia, eclampsia and acute yellow atrophy of the liver. It is regrettable that the country which led the world in the concept that eclampsia was preventable and severe pre-eclampsia could be avoided still has deaths from these two conditions. With adequate ante-natal care pre-eclampsia can usually be detected early and if treated adequately should not be responsible for maternal deaths. Early induction of premature labour is the only sure way of controlling the condition regardless of the period of gestation. Most of the deaths in this group are classified as preventable; the medical management is the major cause and factors relating to the patient make a significant contribution. The actual cause of death is usually a cerebral haemorrhage, coagulation problems and liver failure are the next two causes in order of frequency.

In this group the majority of deaths occur in the teenager in her first pregnancy. This is in contradistinction to some of the other causes of death already discussed. As well as this young age group deaths are found in those women who commence the pregnancy with essential hypertension or chronic renal disease. When pre-eclampsia is superadded the mother's life can be at risk. There are less deaths in this group than previously as these pregnancies are being terminated more frequently these days.

Although not connected with the above group deaths from subarachnoid haemorrhage and cerebrovascular accidents are included. In this group the death is usually unavoidable and unexpected. When added to deaths from cerebral haemorrhage in patients with pre-eclampsia or eclampsia it forms one of the commonest causes of maternal death.

There are other important causes of maternal deaths that are not included in this short paper. These are cardiac disease associated with pregnancy, Caesarean section complications of anaesthesia, ruptured uterus, amniotic fluid and air

embolism suicide traffic accidents and associated neoplasia

Each country will have special problems which may justify a maternal death rate a little higher than her neighbour. In Australia such problems are our distances and our aboriginal population. In any comparison with other countries the abortion rate must also be quoted for in many developed countries most patients at risk have pregnancies terminated or the country has achieved zero population growth and so eliminated the major group at risk: the elderly multigravidae.

Taking all this into consideration there is no doubt that we can reduce the incidence of deaths directly due to pregnancy even more than we have done in the last triennium.

Deaths associated with pregnancy comprise the smaller of the two groups and the number has remained stationary over the nine year period. Most of these deaths are unavoidable and therefore a reduction will be more difficult.

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INHIBITION OF PUERPERAL LACTATION

A Double Blind Study of Bromocriptine and Placebo

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MATERIAL AND METHODS

(a) Patients

Informed consent to take part in the study was sought from parturient patients in Queen Charlotte's Hospital London who did not desire to breast feed. Fifty two patients embarked on the trial but only 36 returned sufficient data for analysis.

(b) Drugs

All patients were randomly allocated indistinguishable capsules of "active" Bromocriptine (2.5 mg) or a pharmacologically inactive placebo to be taken two daily for fourteen days from day 0 (delivery) and one daily for a further seven days.

(c) Data collection

1 *Patients symptoms* After instruction the patients were asked to fill in and return after 28 days data cards (Fig. 1) concerned with milk production, breast engorgement and breast pain.

2 *Blood sampling* 10 ml peripheral venous blood were taken within one hour of delivery (day 0), 48 hours after delivery (day 7) and 7 days post-delivery. After centrifugation plasma was stored at -20°C until assay.

(d) Assay

Assay was performed by the double antibody radioimmunoassay method modified from a method previously used for human placental lactogen (11). After iodination with chloramine T (35 μg for 30 sec) prolactin was purified on a sephadex G100 column (1.5 \times 70 cm). The elution buffer contained 0.1% bovine serum albumen (BSA). The assay buffer contained 2.5% BSA. Incubation continued for three days with the first antibody, one day after addition of labelled prolactin and one day after antirabbit gamma globulin serum. Dilute horse serum (0.1 ml 25% in assay buffer) was added to all assay tubes. With this method no cross reaction between the prolactin anti-serum and HCG (1000 i.u.), LH/FSH (500 i.u.), HPL (32 ng) and growth hormone (63 ng) was found (1).

D tea													
D ya f tr tm t													
P ro ete	1	2	3	4	5	6	7	8	9	10	11	12	13
Milk p ducti (Try E lpatien)													
Co g tl													
Pe													

S e f 4 p into 0 no milk 1 m drops 2 slight outflow 3 stree f ilk
 S of 4 point 4 ab t 1 mild 2 m derote 3 se ere

Comm nts/Sid Eff cts:

Fig 1 Part of patients data card and score sheet

RESULTS

1 Prolactin levels (Fig 2)

At day 0 there was no significant difference in plasma prolactin levels of the two groups whereas by day 2 the level was significantly lower in patients taking Bromocriptine ($p < 0.001$). By day 7 this difference had lessened with descent of the placebo group prolactin level but it was still significant ($p < 0.02$). At six weeks levels were down to normal (shaded) area but the number of samples collected then was insufficient to warrant inclusion.

2 Milk suppression (Table I)

Throughout the 28 days post delivery the active Bromocriptine regime was considered by the patients to be significantly better in suppressing lactation ($p < 0.01$). As 100% suppression at four weeks was reported in the Bromocriptine group no rebound lactation therefore occurred in the week following termination of therapy.

3 Breast congestion (Table II) and breast pain (Table III)

The greatest significant improvement on Bromocriptine of these symptoms when compared to placebo was noted in the first seven days ($p < 0.01$) which is when the most discomfort is reported.

4 Menstrual pattern (Table IV)

There was a definite trend towards earlier return of menses in the active Bromocriptine group although menses were still absent in similar numbers in both groups. This difference may be influenced by the greater number of patients in the placebo group who failed to return for postnatal check up thus forcing their subsequent menstrual history to be classified as unknown.

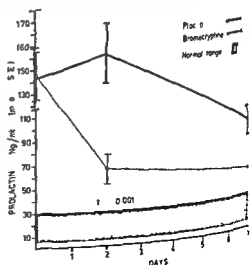


Fig 2 Comparison of prolactin levels for one week post parturition

Table I Milk suppression

Days	14 Days		21 Days		28 Days	
	Active	Placebo	Active	Placebo	Active	Placebo
1/17 (6%)	14/70 (70%)	3/16 (19%)	16/18 (89%)	5/15 (33%)	17/17 (100%)	10/17 (59%)
$p < 0.01$	$p < 0.01$		$p < 0.01$		$p < 0.01$	

DISCUSSION

Parturition lactogenesis is closely related to the secretion of prolactin by the pituitary (13). During pregnancy prolactin secretions have reached a high level but the lactogenic action is opposed by high circulating levels of oestrogen and progesterone of late pregnancy. Dopamine normally inhibits prolactin secretion at pituitary level (14) but pregnancy is overcome by the increased prolactin output from the enlarged prolactin secreting pituitary cells. Hence when the inhibitory effect of prolactin is removed around parturition lactogenesis occurs.

Bromocriptine as a Dopamine agonist inhibits prolactin secretion (15) by its stimulatory effect on dopamine receptors in the hypothalamus thus increasing prolactin inhibitory hormone secretion and perhaps by a local action at pituitary level. Therefore if given at the right time in sufficient quantity Bromocriptine inhibits prolactin secretion and stops lactation.

The thesis is supported by the results of this study for within two days there was a significant falling of serum prolactin levels in patients using bromocriptine when compared to placebo ($p < 0.01$). This significant difference is continued at 7 days ($p < 0.01$) and accounts for milk production in 60% of Bromocriptine patients compared with 6% on placebo and consequent significant differences in pain and congestion ($p < 0.01$).

Bromocriptine is also reported to be of use in suppressing galactopoiesis (5) which also depends on prolactin. Although it is known that prolactin levels can be back to normal within three weeks of parturition (18) a wide fluctuation is noted which may be related to the stimulatory action of the suckling reflex (6). These factors could account for reports of rebound lactation (4, 7) when shorter dosage regimes were used. To overcome this in our study 5 mg daily was given for two weeks followed by 2.5 mg for a further seven days. Results show that 100% of treated patients reported dry breasts one week after cessation of Bromocriptine therapy.

Pain and congestion were not significantly different after the first week but it is of course during this time that pain is most troublesome.

Removal of the gonadal inhibitory effect of hyperprolactinemia causes ovulation in the galactorrhoea amenorrhoea syndrome (19, 20, 21). This can also occur in the parturient patient and makes early conception a distinct possibility (22). Six weeks after delivery menses had returned in 40% of the Bromocriptine group compared with 22.7% on placebo. The high drop-out rate however makes these figures unreliable although note should be taken of the trend.

At the dosage used therefore Bromocriptine is a safe effective suppressor of lactation. Such prolonged therapy does not however lend itself to consumer preference and further studies are needed.

Table II Breast pain relief

Days	14 Days		21 Days		28 Days	
	Active	Placebo	Active	Placebo	Active	Placebo
1/20 (5%)	17/70 (85%)	13/16 (81%)	16/18 (100%)	13/15 (87%)	17/17 (100%)	15/15 (100%)
$p < 0.01$	NS		NS		NS	

Table III Breast congestion relief

7 Days		14 Days		21 Days		28 Days	
Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo
14/20 (70%)	2/17 (12%)	17/20 (85%)	13/16 (81%)	18/18 (100%)	13/15 (87%)	16/17 (94%)	14/15 (93%)
$p < 0.01$		NS		NS		NS	

Table IV Menstrual pattern

Menses	Bromocriptine	Placebo
% returned	40	22.7
% absent	33.3	31.8
% unknown	26.6	45.45

to formulate the ideal regime. Additional work is also necessary to see whether indeed ovulation and menses are brought forward and whether users of these drugs should have contraceptive measures recommended earlier than is usual.

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PROLACTIN RESPONSIVENESS TO TRH IN AMENORRHEIC WOMEN WITH AND WITHOUT GALACTORRHEA

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To study 60 women were given intravenous injection of 0.1 µg TRH to assess its diagnostic potential as a stimulus to PRL release. Following the administration of TRH there was a prompt increase in serum PRL to 6.7 in 34.7 in 24.1% and 34.7% in normal women, amenorrheic patients, non-tumoral galactorrheic cases, and patients with pituitary tumors respectively. The TRH response was a baseline of PRL levels was statistically different in all groups, but the women with pituitary tumors showed a blunted response. The percent of increase of PRL levels after TRH was similar in amenorrheic women regardless the presence or not of galactorrhea; this increase was significant greater than in patients with pituitary tumors ($p < 0.01$). The percent of increase above baseline of PRL was significantly greater in amenorrheic women than in amenorrheic patients with pituitary tumors. In both groups, but there is a diminished PRL secretion after TRH in amenorrheic women and the presence of galactorrhea or hyperprolactinemia. A blunted response to TRH in hyperprolactinemic women can be indicative of a pituitary tumor.

In the intravenous use of 0.1 µg TRH according to our procedure (3). In the group of patients with amenorrhea and galactorrhea, 11 out of the 42 had an enlargement of the sella which was considered compatible with pituitary adenoma. The remaining 19 out of the total series of 60 patients had no amenorrhea (symptoms of hypothyroidism, etc.). In this group the following diagnoses were established: cases of "menopausal" 2 cases of amenorrhea, 3 cases of "prolactinemic amenorrhea" and 4 cases of abnormality in the estrogen feedback mechanism. 5 normal menstruating women were included in this study in order to evaluate their PRL responsiveness to TRH. All women had both clinical and by the laboratory tests a normal thyroid function. Serum PRL was measured by radioimmunoassay according to the method of Fung et al. (4) and amenorrhea, variation in our laboratory has been reported previously (5). Unpaired and paired Student's t -tests were used to compare the results.

RESULTS

Normal women

In the 12 normal women the basal values of PRL ranged from 2 to 13 ng/ml with a mean value (\pm S.E.M.) of 9.2 ± 3.3 (Fig. 1). In response to 0.1 µg TRH, PRL levels had a maximum increase of 9.6 ± 1.7 ng/ml (mean \pm S.E.M.) which was statistically significant ($p < 0.01$). The maximum increase in these six patients showed a range from 36 to 160 ng/ml.

Amenorrheic women with or without galactorrhea

In this group four out of the 60 patients were hyperprolactinemic despite the absence of galactorrhea. Baseline PRL ranged from 2 to 6 ng/ml with a mean value (\pm S.E.M.) of 3.1 ± 3.3 (Fig. 1). TRH injection elicited a mean maximum increase in PRL levels of 6.8 ± 0.9 (\pm S.E.M.), this rise was

used to assess the validity of prolactin (PRL) secretion in response to synthetic thyrotropin releasing hormone (TRH) to differentiate galactorrhea from amenorrhea without pituitary tumors has a well-established reports (1-3). We have previously found that a blunted response to TRH injection in hyperprolactinemic women may be indicative of a pituitary tumor (7). Present investigation was designed to evaluate the TRH-induced PRL secretion in a series of galactorrheic women and hyperprolactinemic patients with amenorrhea of presumed pituitary origin as well as normal menstruating women were included in the study.

MATERIAL AND METHODS

Total of 60 amenorrheic women either with or without galactorrhea were tested in their PRL secretion response

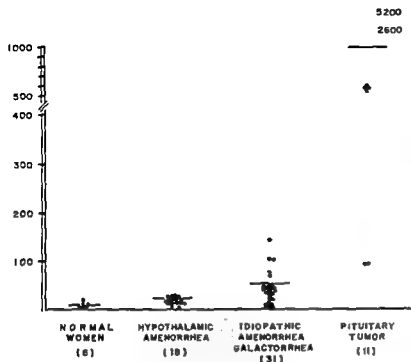


Fig 1 Basal serum PRL are presented on the vertical axis in ng/ml. Mean PRL values represented by horizontal bar was highest in pituitary tumors. Patients with pituitary tumors had the highest PRL concentrations.

statistically significant ($p < 0.001$). The maximum increase ranged from 26 to 170 ng/ml.

Idiopathic amenorrhea galactorrhea

The mean of baseline PRL levels was 54.7 ± 12.2 ng/ml (\pm S.E.M.) and the maximum increase after TRH was 98.4 ± 13.1 ng/ml (mean \pm S.E.) which was significant ($p < 0.01$). PRL increments ranged from 12 to 360 ng/ml. PRL secretion in response to TRH was similar in both groups of amenorrheic women regardless the presence or not of galactorrhea; conversely the amenorrheic women had a lesser PRL secretion after TRH than normal women ($p < 0.001$) as shown in Fig. 2.

Galactorrheic women with pituitary tumor

Women in this group presented the highest basal PRL levels with a range from 90 to 5200 ng/ml; the mean value (\pm S.E.M.) was 1086.5 ± 482.2 ng/ml. Mean maximum increase after TRH was 1382 ± 602.7 (\pm S.E.M.). This response was not statistically significant. The mean maximum increase of PRL above baseline in women with a tumoral process was significantly less ($p < 0.001$) than in both groups of hypothalamic amenorrhea and idiopathic galactorrhea (Fig. 2).

DISCUSSION

In this study of amenorrheic patients we confirm that elevated levels of PRL may occur in the presence of galactorrhea and conversely patients with galactorrhea may be found to have normal PRL levels (2, 6). It was also evident that patients with pituitary tumors presented the highest PRL levels which leads to consider that those cases with PRL values above 100 ng/ml require proper follow up despite the normality of pituitary tomography. The release of PRL in response to TRH in amenorrheic women with or without galactorrhea showing no evidence of pituitary tumor was significantly less than in normal women. Such responses to TRH in patients with hypothalamic or pituitary disturbances have been reported. On the other hand, exaggerated responses to TRH have been reported in patients with hyperprolactinemia (8). However, the studies with the largest number of patients indicate that women having a hypothalamic disturbance expressed clinically by amenorrhea or galactorrhea show less release of PRL after TRH stimulation than normal women. It has been suggested that an "ultra-short feedback effect" of the PRL on the normal prolactotrophes preventing a normal release of PRL could be operative (7) but the demonstration by us that a diminished response to TRH is

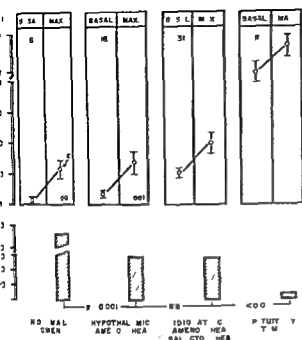


Fig 2 Release of PRL in response to TRH. Responses are expressed as maximum increase above baseline and as per cent increment from the basal values.

ed in amenorrheic women regardless the presence or not of elevated PRL levels weights against the hypothesis.

It seems that patients with amenorrhea-galactorrhea syndrome present a varied response to the stimulating and inhibiting tests of hypothalamic-pituitary function (3). We can conclude from the present study that although basal serum PRL levels do not distinguish women with tumoral process from those with hyperprolactinemia of other etiology, administration of TRH may be helpful in separating patients with pituitary tumors from those with galactorrhea of other causes. This is in agreement with our previous studies (3-9) in which was stated that elevated PRL levels associated with a blunted response to TRH and absent or deficient response of both gonadotropins in response to TRH could be indicative of an underlying pituitary microadenoma.

ACKNOWLEDGEMENTS

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TRIAL OF A NEW ANALOGUE OF LH RH IN NORMAL SUBJECTS AND IN CASES OF ANOVULATORY STERILITY

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Abstract A new and potent analogue of luteinizing hormone releasing hormone (LH RH) the des gly 6-D LH RH which was synthesized in 1975 by Schally's group was assessed in normal volunteers both men and women and in six cases of anovulatory sterility. The new substance seems to be one of the most active and LH releasing factors. Its effect is stronger in the female than in the male acting more actively on LH than on FSH. The magnitude of the effect is greater not only in amount but also in time the levels of gonadotropins after the injection being higher and remaining longer. This last property makes the new substance ideally suitable for the treatment of anovulatory sterility. In six sterile women one ovulated and two became pregnant.

releasing effect on LH and to a lesser extent on FSH secretion by a decapeptide obtained from the hypothalamus of the pig is today very well known through numerous research works in the last seven years. Two years ago Schally's group began to investigate the possible action of analogues of the synthetic decapeptide. The compounds assayed were D ala-6 des gly NH₂ 10-LH-ethylamide (2 5 6 8 10) D lys 6-LH RH des gly 10 pro-9-ethylamide LH RH (3 7) des gly 10 pro-9 propylamide LH RH 3 and D leu des gly NH₂ 10-pro-ethylamide LH RH (9). The research group (3) has investigated the possibility of introducing a D tryptophan group in position 6 in the decapeptide. The so obtained D trypt LH RH was sent to our group for clinical trial at the end of 1975.

MATERIAL AND METHODS

The study is divided into two parts. In the first one the effect of the analogue was compared with that of synthetic

LH RH. Nine normal healthy and menstruating women volunteers—chosen among students and nurses aged from 18 to 26 years—were followed during two previous cycles monitored by temperature charts vaginal cytology and pregnanediol determinations in order to prove their normality. During the late follicular phase (12-14 days) of the third cycle each volunteer was injected with 100 µg of LH RH intravenously and tested for LH and FSH in plasma by double radioimmunoassay against the NIH kit. The samples of blood were taken at zero time and after 15 30 45 60 90 and 120 min.

The same control or pattern curves were obtained from eight normal student boy volunteers ranging from 18-22 years old.

Six girls also volunteers and normal were tested with only 10 µg of D-6-trypt LH RH but in such cases the extraction times were prolonged up to 180 240 360 and 480 min because the first assays showed that its action was delayed.

Another group of young normal women was tested with graduated dosages of the analogue 1 5 7.5 and 10 µg. Finally six normal boys were tested in the same way as the girls. They were also normal students volunteers aged from 19 to 23 years.

The second part of the study concerned sterile anovulatory women. The group was composed of six women aged from 31 to 36 years whose sole cause of infertility was anovulation. Basic requirements for this diagnosis were 1) flat temperature charts for a minimum of four months 2) endometrial biopsies showing monophasic cycle in two consecutive cycles 3) low pregnanediol excretions in the second half of at least three consecutive cycles and 4) absence of other causes of sterility in the couple. All the six women were monitored in a previous cycle with plasma FSH and LH every four days basal temperature and urinary pregnanediol estimations. In a second cycle they were treated with an intravenous infusion of 40 µg of D-6-trypt LH RH during eight hours followed by a further 40 µg intramuscularly. The time of the initiation of the treatment being the 13th day of the cycle. FSH and LH estimations were done before the treatment and 15 30 45 60 90 120 180 240 360 and 480 min after the starting of the infusion.

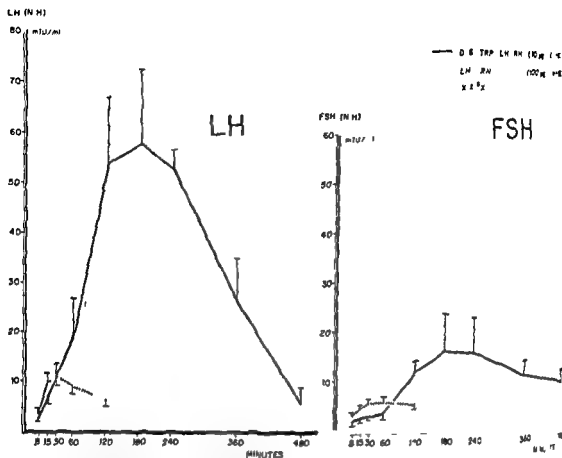


Fig 1 Effect of D-6-trypt LH RH (10 µg) compared with the action of LH RH (100 µg) on LH and FSH levels in plasma of normal young women (preovulatory phase)

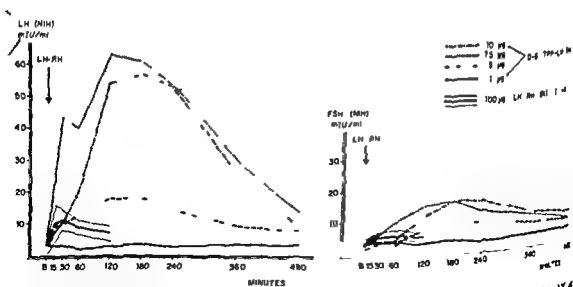


Fig 2 Effect of different dosages of D-6-trypt LH RH on plasma FSH and LH of normal women. The shaded area

shows the oscillations of FSH and LH of LH RH

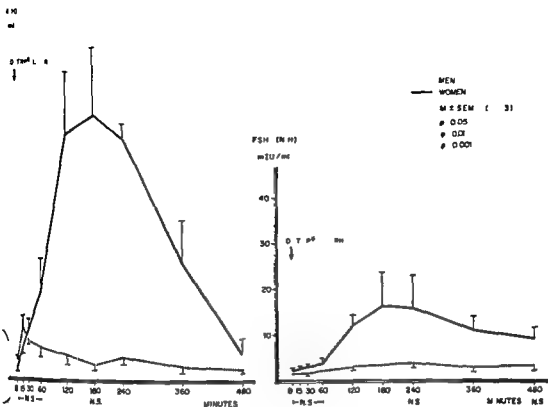


Fig. 3. Comparison of action of D-6-trypt LH RH on plasma LH and FSH levels of men and women.

RESULTS

Results are shown graphically in Figs 1-4. In Fig 1 is seen the effect of 10 μ g of D-6-trypt LH RH intravenously on the plasma levels of LH and FSH. The results in six normal young men were compared with another 9 normal young women injected with a tenfold dose of conventional LH RH. The response of plasma LH is 1% higher with the analogue than with the conventional LH RH. Using also the D-6-trypt LH RH the height of the response is eightfold (800%) compared with LH RH.

The FSH response although not so great is also significantly increased approximately 300% higher and 400% longer. The importance of these differences is very remarkable because there is a general agreement that the pulse of FSH and LH is elicited by current LH RH too short to induce physiological changes at the ovarian level. It should be emphasized that the higher and longer responses

were obtained with only one tenth the dosage of the analogue as compared with the conventional LH RH.

Fig 2 shows the effects of an increasing dosage in individual cases. One microgram has practically no effect but a dosage as small as 5 μ g is able to promote as high but much more prolonged action as 100 μ g of LH RH. With 7.5 and 10 μ g the effects are as described in Fig 1.

In Fig 3 the differences between normal men and women are shown. Six normal young men were compared with six normal young women. In the male the responses are lower both in FSH and in LH. This fact has not been described with conventional LH RH or other analogue compounds. We should call attention to this difference.

Finally six sterile anovulatory women selected as already described were treated with a total amount of 80 μ g of D-6-trypt LH RH. The outline of the clinical experiment was described above. It should be emphasized that in all cases the

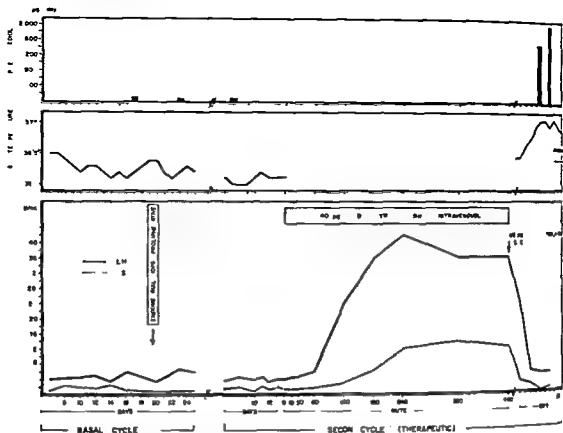


Fig 4 Pregnandiol basal temperature FSH and LH during two cycles in a sterile anovulatory woman. In the second cycle there was injected a total amount of 80 μ g

D-6-trypt LH RH partly intravenous and partly intramuscular. The woman became pregnant in the third treatment cycle.

tryptophan analogue was employed alone without any previous treatment with HMG, HGC or Clomiphene. Of the six women, two became pregnant and in one more ovulation definitely occurred. In the two pregnancies, one resulted in delivery of a healthy child and the other in an abortion. In Fig 4 the results from the first pregnancy are shown.

COMMENTS

The discovery and synthesis of porcine LH RH was followed by a great deal of hope in the successful treatment of anovulatory sterility and (or) amenorrhea. However, the results in clinical practice have not confirmed this preliminary expectation. This was in part due to the short discharge of LH that is commonly obtained with the current dosages of the decapeptide. The peak of the LH response to 100–200 μ g takes place for no longer than 30–60 min. It seems that such a short stimula-

tion is not enough to provoke ovulation and correct luteinization that must follow the export of the egg. The great advantage of the analogue we are now using is not only its stronger action but the much longer effect. Up to now we have only very limited experience but we believe that this substance if associated with follicle stimulation, either HMG, Clomiphene or both, may be an important tool in the treatment of ovulatory disturbances in women.

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liquidum acidum benzilicum butylhydroxyanisolum

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Egenskaper DAKTAR vaginalkram innehåller som aktiv substans miconazoli, ett imidazolderivat. Miconazoli har såväl in vitro som in vivo fungicid effekt mot flera av de vanligaste svamparter.

DAKTAR absorberas i mycket ringa grad vid lokal applikation.

DAKTAR har god kosmetiska egenskaper och innehåller ej lanolin eller parabener. I ett fall av sensibilitet bör svampsteras. Krämen färgar ej kläderna och kan avtvättas med tvål och vatten.

Klinik DAKTAR är effektiv vid vaginitter orsakade av Candida spp. och andra svampar.

ter. Så alltså, i ganska många kliniska utvärderingar har konstaterats i ca 90% av de behandlade fallen. Vid behandling med DAKTAR erhålls en snabbt inträttande effekt med snabb förändring av symtomen som sveda, klåda och flulor. Behandling med DAKTAR sänker förhöjda vaginala pH-värden, vilket har en gynnsam effekt på den naturliga bakteriefloran. Tillväxt DAKTAR lindrar sig väl för behandling av symtomen i graviditet och efter födseln. Symtomen i graviditet och efter födseln har en negativ effekt på barnet.

Indikationer Vaginit och vulviter orsakade av svampar.

Biverkningar I sällsynta fall (mindre än 1%) har läkemedlet orsakat irritation i svampsteras.

Dosering En fullständig applikation (5 g kräm 100 mg miconazoli) toms djup i slutet av vardagen. Behandlingen upprepas dagligen i 14 dagar. Det är viktigt för ett gott behandlingsresultat att patienten genomför hela behandlingen. Inget uppehåll behövs göras under menstruation. Vid graviditet bör applikationen toms långsamt och mindre djupa i slutet av applikation och brukas i en medföljande varje förpackning.

Förpackningar Tub 78 g med applikator.



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DANAZOL AN ANTIGONADOTROPHIC AGENT IN THE TREATMENT OF RECURRENT PELVIC AND INTESTINAL ENDOMETRIOSIS

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Six cases of pelvic endometriosis were treated with Danazol 400 mg daily for six months. In four of these cases intestinal endometriosis of varying severity was present. The initial results were very satisfactory. In one case with adenomyosis and continuous spotting, follow-up after cessation of treatment suggests that in some cases a further period of treatment will be necessary.

The results from detailed metabolic and endocrine studies have been reported previously (1, 4, 6, 7, 9, 11) and no further attention was paid to these aspects.

PATIENTS

The series includes six cases with pelvic endometriosis of which four had varying degrees of additional involvement of the intestinal tract.

Case 1

A 37-year-old woman had suffered from irregular periods, intermenstrual spotting, dysmenorrhoea, dyspareunia and primary infertility and she had undergone a laparotomy in 1974. The uterus contained a swelling, possibly an intramural fibroid, while the peritoneum was sprinkled with endometriotic implants and the pouch of Douglas was closed by endometriotic tissue. Involvement of both ovaries (diameter 4-5 cm) and also the serosa of the rectum was apparent. The swelling was excised from the anterior uterine wall and both ovaries resected. Both specimens showed typical endometriosis.

Treatment with a variety of hormonal preparations was followed by an unsatisfactory response.

In July 1975 treatment by Danazol was started because of dysmenorrhoea, dyspareunia, lower abdominal pain during the latter half of the period and irregular periods with intermenstrual spotting. The right ovary was 5-6 cm in diameter and the uterus and the left ovary almost normal.

Six weeks later the symptoms had almost disappeared apart from spotting. The uterus was now retroverted, both ovaries normal and not tender. Following treatment for three months a curettage was performed and subsequently a hysterectomy because of the continuous spotting throughout three years. The ovaries were small and adherent, the pouch of Douglas closed by fibrous tissue. A number of serosal adhesions were found but no visible endometriotic implants were present in the serosa.

Follow-up (14 months). The ovarian endometriosis recurred after 17 months. Treatment with Danazol was resumed with a satisfactory response.

METHODS

Following a study of the literature we decided to administer Danazol orally in a dose of 400 mg twice daily for six months. The patients were seen monthly for clinical as-

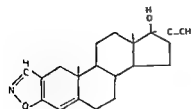


Fig 1 Chemical structure of Danazol

Case 2

A 28 year-old woman who had a curettage in 1971 for irregular periods. Her uterus at that time was comparable to that seen in an 8 weeks pregnancy. In 1972 both ovaries were resected being about 8–10 cm in diameter. Diagnosis: endometriosis.

In subsequent years variable symptoms of lower abdominal pains during the luteal phase, dysmenorrhoea, dyspareunia, menorrhagia and primary sterility were reported. Various types of hormonal treatment failed.

When Danazol was started in October 1975 the main symptoms were menorrhagia (periods about ten days duration) and pronounced dysmenorrhoea. The uterus was slightly enlarged, the right ovary 4–5 cm in diameter and a typical involvement of the pouch of Douglas was present at that time. During the six months treatment the right ovary diminished to almost normal size and the uterosacral area became fibrotic and not tender.

During the follow up period (12 months) no complaints apart from pronounced pain produced by bowel movement the first few months when a typical small mass was present behind the cervix. She conceived January 1977 following primary sterility for 8 years.

Case 3

A 24 year-old woman was admitted to this hospital in December 1975 with subacute lower abdominal complaints preceded by dyspareunia and vague lower abdominal pain for some months. At laparotomy the right ovary was normal apart from endometriotic foci. The left ovary

was enlarged (diameter 8 cm) with endometriotic foci. The uterus was normal. The left ovary, the abdominal part of the left tube and the rectum formed a dense partially fibrotic plate of endometriotic tissue invading the rectal wall which was almost frozen to the osseous pelvis. The abdomen was closed taking into consideration the fact that the patient had no actual intestinal complaints.

Treatment with Danazol was started immediately and the pains disappeared quickly. Six weeks later the mass on the left side had diminished considerably and the tenderness had disappeared. When the treatment was withdrawn the pelvic organs were normal by vaginal examination apart from tiny fibrotic nodules in the uterosacral region.

During a follow up period of two months a tender mass, diameter 4–5 cm was found in the left side. Moderate lower abdominal pains were still present but no other intestinal symptoms. Treatment by Danazol was resumed and in the ensuing month the mass definitely reduced in size and was no longer tender. No other complaints were elicited.

Treatment by Danazol is continuing.

Case 4

A 22 year old woman had a left-sided oophorectomy due to endometriosis in 1971, followed by treatment with a variety of hormonal

agents. Referred to this hospital in 1974 because of recurrent rectal bleeding for 18 months. She presented with abdominal pain and dysmenorrhoea. The retroperitoneum and the retrouterine pouch showed a gradually fibrotic infiltration with the rectal wall thickened. By proctoscopy an area with polyps was found. The biopsy showed atypical polyps probably endometrial.

Further attempts with other hormonal treatment failed. In March 1976 treatment with Danazol was started. The patient had at that time a mass behind the uterus, about 5 cm in diameter which probably included the right ovary. Four weeks later the pains and the rectal bleeding disappeared, the bowel function being perfect, and the mass no longer tender. When treatment with Danazol was withdrawn in October 1976 the uterus retroverted with small gritty areas in the pouch of Douglas and slightly fibrotic utero-sacral ligaments.

Follow up (6 months). No complaints. The vaginal examination as mentioned above.

Case 5

A 36-year old woman was admitted to this hospital because of complaints of secondary infertility and dysmenorrhoea. Due to cornual occlusion an implantation of the tube was performed. The tissue removed showed an inflammatory reaction and no signs of endometriosis.

In 1971 a right sided hemicolectomy due to endometriosis of the caecum was performed. After this the patient was followed in the out patient department of this hospital complaining of menorrhagia, dysmenorrhoea, dyspareunia, secondary infertility and persistent bowel problems (three motions a day). Hormonal treatment of various types failed.

When treatment by Danazol was started in November 1975 the ovaries were about 8 cm in diameter and the uterus slightly enlarged. After a few weeks the pain and tenderness disappeared. When treatment was discontinued after six months the ovaries were normal.

During a follow up period of 11 months there were no complaints, the periods being normal with no dysmenorrhoea. Dyspareunia was not reported and the patient had two normal bowel movements a day. The uterus and ovaries were normal but a minimal amount of fibrotic tissue remained behind the cervix.

Case 6

A 30-year old woman had a laparotomy performed elsewhere which showed the right ovary containing a haemorrhagic (follicular) cyst which was removed. The left ovary was normal. On the caecum could be seen an endometriotic nodule (diameter 1 cm).

In 1975 a second laparotomy (performed elsewhere) showed the left ovary to be cystic due to endometriosis and this was resected. Disseminated endometriotic nodules were observed on the pelvic peritoneum. The rectum was



(a) Uterine endometrium and adenomyosis. Con- discrepancy between the epithelium, the glands the stroma of the endometrium and of the adenomyo- $\times 25$ (b) Endometrium. Moderate atrophy of the glands, rather flat epithelium and ation of the stroma with fibrosis and very few vessels

(high power $\times 250$) (c) Adenomyosis. Almost normal fol- licular phase with straight glands, tall epithelium, pro- liferating glands and numerous vessels (high power $\times 750$) (H. Sogaard, The University Institute of Patho- logy, Aarhus)

ly adherent to the left uterine margin and showed an ing endometriotic mass which included the rectal

with Danazol was started in June 1976 when included lower abdominal pain together with produced by bowel motion and dyspareunia. Vaginal on showed the uterus itself to be normal but with tended tender mass between the uterus and the rec

uing two months of treatment the mass had re- sed considerably, no tenderness and almost no pain- asted. After five months of treatment (November) no symptoms were reported and the mass was now fibrotic and not tender. follow-up (3 months). Slight lower abdominal pain. The to vary 3-4 cm, not tender. No treatment necessary at moment.

RESULTS AND DISCUSSION

The role of Danazol in the treatment of gynaecologi- cal disease is still the subject of continuing re- search. Thus far convincing preliminary results have been reported mainly in endometriosis (3, 5, 6, 8) but even in this condition the indications for treatment are not fully defined.

The results from the treatment of endometriosis with Danazol are apparently dependent on the dose and duration of treatment. As far as the former is concerned, doses of 100-800 mg daily have been used. The extent of this disease and the symp- tomatology should be used as an initial guide but the deciding factor must be the clinical response as

measured by the symptomatic improvement and the reaction of the endometrial process. At the same time attention must be paid to the type and degree of side effects although these are always mild (3). The object of treatment should be a regression of the disease process to such an extent that permanent cure is obtained.

In the small series presented a daily dose of 400 mg was used representing an average of the doses used in the reports previously available. The duration of the treatment was six months although published studies have reported treatment periods of between three and twenty one months.

The initial results were generally satisfactory. All six were relieved of pain in two to four weeks. A patient presenting a history of rectal bleeding for 4 years (case 4) was cured from this symptom in four weeks. The endometrial masses regressed quickly and tenderness disappeared. After three months (case 1) five months (case 6) and six months (cases 2, 3, 4, 5) the extrauterine process had regressed to a very moderate or insignificant amount of scar tissue with no tenderness.

It should be noticed that Danazol did not suppress the adenomyosis (Fig. 2) occurring in case 1 in spite of the excellent response to treatment in the endometrium and the extrauterine mass. A similar observation was published by Lauenstein et al. (8).

Side effects were mainly an increase in weight of one to six kg with or without clinical oedema. Two cases had a fairly normal menstrual bleeding a few weeks after treatment was started and while episodes of vaginal staining, spotting or slight bleeding at irregular intervals during treatment may be recorded as a side effect this was only a minor complaint in one patient (case 1). Hot flushes, dizziness and skin oiliness occurred as a minor temporary complaint in one case.

So far the initial results from treatment with Danazol in genital endometriosis outside the uterus and in endometriosis involving the bowel to a varying extent were satisfactory. Bearing in mind that the series included cases persisting following surgery and cases treated by various steroids before Danazol was employed.

The crux of the matter is the long term prognosis and so the patients must be followed for a number of years. The extent to which treatment with Danazol is able to eliminate active disease is still to be seen. The literature presents little evidence about the course of the disease following cessation of Danazol treatment (2).

The preliminary results from a ten year follow up indicate that further treatment may be necessary for some and probably all cases. If treatment may be used intermittently in order to be less permanently aiming at the lowest amount of Danazol producing the desired effect.

It may be concluded that Danazol is able to suppress the symptoms produced by active endometriosis and to induce an inactive phase of endometrial tissue in the uterine appendages, the serosa and the bowel. The demand for long term treatment of such cases is the subject of future clinical research in several centres.

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ESTRIOL CONCENTRATIONS IN URINE AND SERUM
IN PATIENTS WITH VARIOUS INTESTINAL DISEASES

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Pregnant women with ulcerative colitis shows low estradiol values in both serum and urine. 18 patients with ulcerative colitis, 4 patients with Crohn's disease and 3 patients with a bypass operation were examined. Low estradiol concentrations were seen only in the patients with ulcerative colitis, especially in patients where an operation has been performed. In one patient with severe ulcerative colitis the estradiol concentration in serum was low until intestinal function normalised and the estradiol concentration went up exactly when the diarrhea stopped. However, no unequivocal connection between low estradiol concentrations and diarrhea could be demonstrated.

We have several times observed that pregnant women with ulcerative colitis frequently have low estradiol values during an otherwise uneventful pregnancy and we have therefore found it adequate to examine a group of pregnant women with various intestinal diseases, particularly in view of the fact that as far as we know, no such study has so far been published.

MATERIALS AND METHODS

The study involves 25 patients admitted to the hospital during the period 1974-76. We have an average of seven urine analyses and four blood analyses per patient and Table I is showing the results of the patient material. Figures in brackets indicate the number of patients in individual groups who had several successive urine analyses falling below the lower limit of the reference area ($\bar{x} - 2$ S.D.). All patients with Crohn's disease and bypass operations showed normal estradiol values despite the fact that one of the patients with bypass operation had diarrhea during her pregnancy. Table II gives a more specified account of the 18 patients with ulcerative colitis. By the term "low estradiol" is meant estradiol concentrations in serum and urine below the lower limit of the reference area. Group A comprises five non-operated patients with low

concentrations of estradiol in urine and serum. Four of the patients had placental insufficiency which was ascertained both on the basis of determinations of the placental lactogen hormone (hPL) and of the children being all "small for date" (1300-1790 g). The last patient in this group had periods with diarrhea and also periods of normal stools during her pregnancy. This patient revealed normal hPL-concentrations all the time and this case will be discussed later on.

Group B consists of seven non-operated patients with normal estradiol concentrations in both urine and serum and they also had normal hPL concentrations in the serum. One of the patients had diarrhea during her pregnancy while the remaining six patients had normal stools. All the patients delivered normal babies on time and the weight of the babies was normal.

Group C contains the six operated patients, four of whom had ileostomy, one patient hemicolectomy and one ileorectal anastomosis. The last mentioned patient had loose stools four or five times daily while the hemicolectomy patient had pulpy stools twice daily per anum. None of the patients had mucous or blood stools. All patients in this group revealed normal hPL-concentrations in serum but low estradiol concentrations in serum and urine. All delivered normal babies (3380-3800 g) on time. Fig. 1 shows the urinary estradiol concentrations in group C as compared with group B.

Methods. Urinary estradiol was measured by a colorimetric method described by Aasted Frandsen (1). Estradiol concentrations in serum was measured by a radioimmunoassay using 125 I-estradiol. The method will be described in detail elsewhere (2). Placental lactogen hormone (hPL) was estimated in serum samples by an immunoelectrophoretic method described by Nørgaard Pedersen & Gaede (3).

RESULTS

In the patients with Crohn's disease or by-pass operation we observed normal estradiol concentrations both in serum and urine. Of 18 patients with ulcerative colitis, four had placental insufficiency estimated on the basis of low hPL-concentrations in serum and

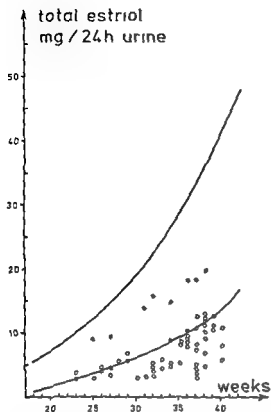


Fig 1 The urinary estriol secretion in patients with ulcerative colitis. The open circles show the estriol values in six operated patients (group C) while the black circles indicate the estriol values in seven non operated patients (group B)

small for date children. Of the remaining 14 patients seven had low estriol concentrations in both serum and urine. Six of these patients had been operated as described under group C in Materials while the last patient had periods of both low and normal estriol values as shown in Fig 2. It is interesting to notice that in this patient the estriol concentration went up exactly at the same time as the intestinal function normalised and the diarrhea stopped.

On the other hand two of the patients with normal estriol values had periods of violent diarrhea and

Table I The distribution of the patient material

The figures in brackets indicate the number of patients with low estriol excretion in urine

	No operation	Operation
Ulcerative colitis	12 (5)	6 (6)
Mb Crohn	3 (0)	1 (0)
By pass operation	0	3 (0)

Table II The distribution of the 18 patients with ulcerative colitis

The term low estriol means estriol concentration in serum and urine below the lower limit of the normal area

	No operation		Operation	
	Low estriol	Normal estriol	Low estriol	Normal estriol
Number of patients	5	7	6	0
Group	A	B	C	D

thus there seems to be no unequivocal connection between low estriol concentrations and diarrhea in these patients. It should be added that none of the patients with low estriol values received medical treatment.

DISCUSSION

The six operated ulcerative colitis patients with low estriol concentrations had all had a large part of

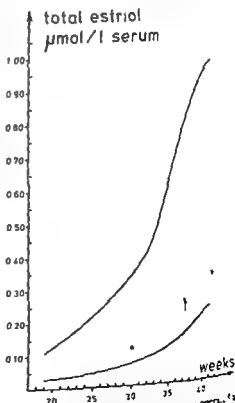


Fig 2 The estriol concentration in a non-operated ulcerative colitis patient showing a clear increase in estriol concentration at the same time as the patient's diarrhea spontaneously stopped (at the arrow)

n removed. No part of the colon had been re-
ed from the three by-pass operated patients.
the operated patient with mb Crohn had only
5-8 cm of her colon removed—corresponding
to the proximal part. Thus, particularly patients
with a large part of colon removed seem to reveal
estrinol concentrations in serum and urine
which may be due to a failure in the resorption of

In this connection it should be added that
Lund et al. (3) observed that two out of three
penicillin-treated pregnant women showed a lower
concentration of estrinol in the urine during the
period of treatment than before. Thus, the authors
believe this is caused by an interruption of the bacterial
conjugation of estrinol glucuronide, which means
that this compound is excreted in the faeces.

In one non-operated patient (Fig. 2) is seen a
close connection between the patient's estrinol con-
centration in serum and urine and her diarrhea.
The normalization of her intestinal function gave a
marked increase in the estrinol concentration. Strong
diarrhea may thus in some patients give low estrinol

values in serum and urine, while in other patients
we have seen normal estrinol values, even in periods
of strong diarrhea. Furthermore, it is noticed that
the hPL-concentration in serum is completely inde-
pendent of both intestinal function and operation.

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TREATMENT OF CLIMACTERIC AND POSTMENOPAUSAL WOMEN WITH 17β OESTRADIOL AND NORETHISTERONE ACETATE

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Abstract Thirty four climacteric and 175 postmenopausal women were treated with three different preparations containing microcrystalline 17β oestradiol and norethisterone acetate for climacteric symptoms and symptoms of oestrogen deficiency. 56 of the women (mean age 57 ± 5 years) were treated with Insekvens® (Group I), 131 women (mean age 53 ± 6 years) with Insekvens® forte (Group II) and 2 women (mean age 55 ± 5 years) with Estrofem® forte (Group III). The patients were followed for 1-3 years. All patients were subjected to a general and a gynaecological examination. Two or more serum samples were obtained from 145 of the patients during the treatment. Laboratory investigations included: Autochemist® analysis of serum levels of triglycerides, cholesterol, Ca, Na, K⁺, alkaline phosphatase, creatinine, glucose, protein, albumin, haaptoglobin, zinc, ferritin, Fe, TIBC, bilirubin, ALAT and ASAT and a radioimmunoassay of serum levels of FSH, LH and low density lipoproteins (LDL).

S-cholesterol levels fell during the treatment in all three groups and in patients with elevated S-cholesterol levels the values were reverted to normal after 6 months treatment. There was no change in triglycerides except in group I where a slight increase ($P < 0.01$) was observed 14 months treatment. There were no other changes in the values obtained from the Autochemist® analysis. Levels of FSH and LH decreased during the treatment and this decrease was more pronounced in groups II and III. Serum LPE levels increased in group I for climacteric women to values observed during a normal proliferative phase and in postmenopausal women to values during the proliferative phase. In groups II and III serum LPE values in postmenopausal women increased to levels corresponding to the luteal phase. Large individual variations in serum LPE levels were observed even during a rigorously standardized tablet intake which might be explained by individual differences in absorption.

The therapy resulted in a disappearance of the climacteric symptoms (vasomotor symptoms, nervousness, irritability and sleep disturbances) and a considerable improvement of the symptoms caused by oestrogen deficiency: atrophic changes in the vulva, the vaginal mucosa, urethra and bladder, and the state of the skin and

skeletal mineral deposits. Few side effects were observed. 34 of the patients discontinued their treatment with the oestrogen preparations, 14 changed to another type of oestrogen and 20 stopped completely. 2 of them due to the discovery of mammary carcinoma. In general the oestrogen preparations tested were well tolerated. The oestrogen dose used in Insekvens® forte and Estrofem® forte seems to be sufficient for an adequate oestrogen replacement therapy in climacteric and postmenopausal women.

The climacteric is a long part of a woman's life and can last over 20 years. The reduction in oestrogen output which takes place during this period leads to a decrease in all oestrogen-dependent functions. Over the years a gradual diminution of the amount of follicles in the ovaries takes place and at last only a few primordial follicles and differentiated follicles remain. An earlier well functioning interaction between the hypothalamus, the pituitary and the ovaries is disturbed by the decreasing ability of the ovaries to respond to gonadotropic stimulation. After the menopause there is an increased production of FSH and LH from the pituitary caused by the lack of a negative feedback of oestrogens upon the hypothalamus. This increase in pituitary gonadotropin production starts even before the cessation of the ovulatory function (1).

The ovaries of women more than two years after the menopause secrete insignificant quantities of oestrogens, but substantial amounts of androgens. Oestrone becomes the major circulating oestrogen and arises almost entirely from the peripheral conversion of androstenedione (for a review, see (1)). The levels of 17β -oestradiol, oestrone and oestrone sulphate in peripheral blood from postmenopausal women are even lower than in males of correspond-

ing age (9). This dramatic reduction in oestrogen brings about somatic as well as psychological troubles which however in many cases can be cured or improved by oestrogen replacement therapy.

The symptoms appearing during the climacteric and postmenopausally can be divided into two groups. The first group includes *climacteric symptoms* depending upon hormonal disturbance. Irregular uterine bleeding. Vasomotoric symptoms (e.g. hot flushes, sweats and tachycardia). Nervousness, irritability, insomnia and depression. The second group consists of *symptoms caused by the cessation of oestrogen production* and which may be regarded as symptoms of oestrogen deficiency. Atrophy of the vulva. Atrophy of the vaginal mucosa. Atrophic changes in the urethra and bladder causing disposition to urethritis, cystitis and urge incontinence and also stress incontinence caused by atrophic changes in the surrounding tissues. Thinning of the skin due to epidermal atrophy and reduced mitosis. Osteoporosis and Changes in blood lipids.

Hormonal Therapy

Oestrogen therapy has been used since the 1930s. Irregular uterine bleeding during the climacteric depends upon the effect of unopposed oestrogens on the endometrium causing prolonged withdrawal bleeding. Administration of progestagens transforms the endometrium to a secretory phase. When oestrogen production decreases the administration of progestagens has no effect and then oestrogen treatment can be added. The vasomotor symptoms appear as a result of adequate oestrogen therapy.

There has been much discussion as to whether nervousness, irritability and depression appear as a result of hormonal disturbance during the climacteric. Other factors probably play a part in the development of such symptoms. Between the ages 50 and 60 years a woman's life often changes course. The climacteric can be considered as a time of losses and women have feelings of inadequacy which can be the cause of the psychological symptoms nevertheless improvement of psychological symptoms as a result of oestrogen treatment have been reported (8, 13, 14).

The symptoms caused by cessation of the oestrogen production show a clearly positive response to oestrogen replacement therapy. A striking effect is observed upon the mucosa of the vagina, urethra and bladder neck. Long standing

urethritis and cystitis disappear following the use of adequate oestrogen therapy. Oestrogen is known to influence the skin by inducing exfoliation, water retention, stimulating hyaluronidase activity and improving skin circulation (7, 17, 25).

Concerning osteoporosis, oestrogen therapy delays and prevents bone loss in postmenopausal women (10, 23).

The influence of oestrogens upon blood lipids has been frequently discussed. Synthetic oestrogens influence the lipid metabolism in several ways. Diethylstilbestrol increases the concentration of triglycerides and decreases the concentration of antithrombin III (6) and changes the levels of coagulation factors VII, IX and X (4). The effect of so-called natural oestrogens on the lipid metabolism is different in this respect: they decrease the serum lipid levels (18) and in addition Åstedt & Jönvall (28) demonstrated that they do not reduce the fibrinolytic activity in the venous wall, which is the case when synthetic oestrogens are used. It is therefore preferable to use natural oestrogens for the replacement therapy in climacteric and postmenopausal women. There is however a need for more controlled studies on the influence of various types of oestrogens in different dose levels upon the postmenopausal lipid and lipoprotein metabolism.

The following types of oestrogens have been established in Scandinavia for replacement therapy. Synthetic steroid oestrogens (ethinyl oestradiol and mestranol). Esterified natural oestrogens (17 β oestradiol valerate). Conjugated natural oestrogens (mainly oestrone sulphate) and synthetic nonsteroidal oestrogens (diethylstilboestrol). Earlier attempts to use free natural oestrogens (17 β oestradiol) in oral preparations proved unsatisfactory mainly due to poor absorption, however 17 β oestradiol in its micronized form has been shown to be active when taken orally (17, 21). Lethbridge & co-workers (22) demonstrated that oral administration of 1 mg 17 β oestradiol + 0.5 mg of oestriol to postmenopausal women increased the plasma 17 β -oestradiol levels to values comparable to those seen in the midcycle phase. Yen and co-workers (27) measured the serum levels of oestrone, 17 β -oestradiol, FSH and LH after intake of one single tablet containing 1 mg of micronized 17 β oestradiol. The levels of LH and FSH were significantly decreased within 3 and 6 hours respectively and both gonadotrophins remained suppressed for 24 hours after the intake. The level of oestrone reached a peak value 6 hours

rising to a 7000% increase after 11 hours and the level of 17β -oestradiol reached a maximum after 5 hours and remained significantly elevated 8 hours after tablet intake. These experiments clearly indicate that micronized 17β oestradiol is readily absorbed and that a significant fraction of the hormone is converted to oestrone.

Continuous treatment with oestrogens is not suitable because it leads to a hyperplasia of the endometrium which sometimes can be difficult to distinguish from an early endometrial cancer. Oestrogen administration should therefore be given—or better combined with progestagen therapy in the second half of the cycle. Progestagens modify the stimulatory effect on the endometrium of continuous oestrogen administration. Progestagen administration given at regular intervals in oestrogen treated patients transforms the endometrium to a secretory phase, prevents atypia of the nuclei and brings about withdrawal bleeding. The present communication describes the clinical trials of a recently developed oestrogen replacement preparation containing micronized 17β oestradiol and oestrol with the addition during the second half of the treatment cycle of norethisterone acetate as the progestagen. **Trisekvens®** NOVO Industri AB Malmö, Sweden.

(1) *Trisekvens®*

- 12 tablets with 2 mg of 17β oestradiol and 1 mg of oestrol
- 10 tablets with 2 mg of 17β oestradiol, 1 mg of oestrol and 1 mg of norethisterone acetate
- 6 tablets with 1 mg of 17β oestradiol and 0.5 mg of oestrol

(2) *Trisekvens® forte*

- 12 tablets with 4 mg of 17β -oestradiol and 2 mg of oestrol
- 10 tablets with 4 mg of 17β oestradiol, 2 mg of oestrol and 1 mg of norethisterone acetate
- 6 tablets with 1 mg of 17β oestradiol and 0.5 mg of oestrol

(3) *Estrofem® forte*

- 28 tablets with 4 mg of 17β oestradiol and 2 mg of oestrol

Clinical Material

The investigation included 209 women who have been followed during 2.5–3.5 years. The clinical material was divided into three groups.

Group I *Trisekvens®* 56 women aged 46–64 years (mean 55 ± 5.7) of which 27 were menopausal. The remaining 29 were still menstruating but had irregular menses and climacteric symptoms such as sweats, hot flushes, tachycardia and depression.

Group II *Trisekvens® forte* 131 women aged 47–69 years (mean 53 ± 6.3). All of this group were menopausal except 7 who still menstruated and 3 who suffered from primary amenorrhoea.

Group III *Estrofem® forte* 22 women aged 49–68 years (mean 55.3 ± 5.2) all menopausal and all of them had undergone hysterectomy before the treatment started.

Before the medication started all women had a general and gynaecological examination, vaginal smears were taken and their breasts were examined. The patients were re-examined once a year. Serum samples were taken prior to treatment and it was intended to repeat this sampling after 6, 12, 18 and 24 months of treatment. It was not possible to obtain serum samples from all patients on every occasion. Samples were obtained from 145 patients (69.3%) twice or more during the period of treatment: 37 in group I, 95 in group II and 18 in group III. All tests were made on fasting samples.

General Clinical Chemical Analysis

S-Cholesterol (24), S-triglycerides (%), S-Ca, Na^+ , K^+ , Na^+ , S-alkaline phosphatase, H^+ Creatinine, S-Glucose, H^+ Protein, S-Albumin, S-Haptoglobin, S-Zinc sulphate, S-Fe, S-TIBC, S-Bilirubin, S-Alanine aminotransferase (S-ALAT) and S-Aspartate aminotransferase (S-ASAT) were determined in an Autochemist® automatic analyser at the department of clinical chemistry, St Erik's Hospital, Stockholm.

Hormone Analyses

The hormone assays were carried out at the hormone laboratory, Sabbatsberg Hospital. S-LPE (low polar

Abbreviations and Trivial Names

cholesterol 5 cholesten 3β ol Diethyl stilboestrol (di- p -hydroxyphenyl) 1,2 diethyl ethylene Eth 17β oestradiol 17α ethinyl 1,3,5 (10) oestratriene 17β -diol FSH Follicle stimulating hormone LH Luteinizing hormone LPE Low polar oestrogens (synonym immunoreactive oestrogens 17β oestradiol + oestrone) Mestranol 3-methoxy 17α ethinyl 1,3,5 (10)-oestratriene 17β ol Norethisterone acetate 17β acetoxy 17α ethinyl-4 oestren 3 17β oestradiol 1,3,5 (10)-oestratriene 3 17β oestrone 1,3,5 (10) oestratriene 3 16α 17β oestrone 3-hydroxy 1,3,5 (10) oestratriene Oestrone sulphate 3-sulphoxy 1,3,5 (10) oestratriene 17-one Progesterone 4-pregnene 3,20-dione

MATERIAL AND METHODS

Pharmaceutical Preparations

Three kinds of oestrogen preparations were used in the following dose regimens:

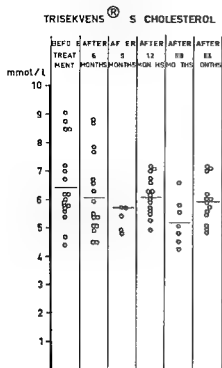


Fig 1 S-cholesterol levels following treatment with Trisekvens®

oestrogens) were determined by the radioimmunoassay technique of Edqvist & Johansson (5) using an antiserum against 17β -oestradiol hemisuccinate bovine serum albumin (22). This antibody reacts to 100% with 17β -oestradiol and to 50% with oestrone (20) and the values are expressed as pmol immunoreactive 17β -oestradiol equivalents per litre. S-FSH and S-LH were determined radioimmunologically using DASP® separation (Organon Oss, Holland). Antisera against FSH and LH and purified FSH and LH for ^{125}I labelling were from KABI AB, Stockholm, Sweden. The values are expressed in units per litre serum of Human Pituitary FSH 68/39 and Human Pituitary LH or ICSH 68/40 respectively.

RESULTS

With the exception of S-cholesterol and S-triglycerides no changes were noted in the values from the general clinical chemical analyses during the treatment. An account will therefore be given only for the values of S-cholesterol, S-triglycerides, S-FSH, S-LH and S-LPE.

Group 1 Trisekvens®

The values for S-cholesterol were normal before treatment except in 4 cases who had elevated levels (Fig 1). The high values fell rapidly to normal

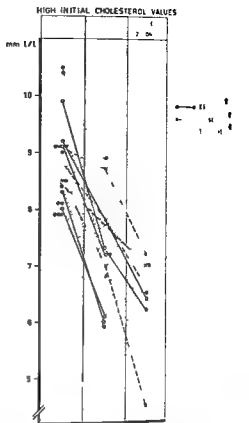


Fig 2 Effect of oestrogen therapy upon S-cholesterol levels in patients with high initial values

levels during treatment (Fig 2). All other values remained normal during the treatment. After 12 months a slight decrease was noted ($P < 0.05$).

The levels of S-triglycerides were normal in all cases (Fig 3) and underwent no changes up to 12 months of treatment. A slight increase ($P < 0.05$) was observed after 24 months.

The levels of FSH and LH decreased ($P < 0.05$) and LPE increased ($P < 0.01$) during the treatment (Figs 4 and 5). There were differences between the menopausal patients and those who were still menstruating when considering the hormone levels. The latter group showed higher LPE values than the menopausal patients (Fig 5). During the treatment, LPE values in the climacteric group corresponded to those found during the luteal phase of a normal menstrual cycle, while the values found for the menopausal group corresponded to follicular phase levels. FSH and LH decreased in both groups, but not to values found during the reproductive phase of life.

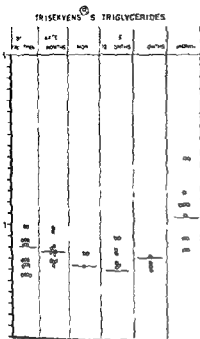


Fig 3 S-Triacylglyceride levels following treatment with Trisekvens®

Group II Trisekvens® forte

There is a statistically significant ($P < 0.001$) decrease in S-cholesterol levels after 12 and 24 months treatment (Fig 6). Those patients who had elevated cholesterol values developed normal levels after 6 months of treatment (Fig 2). S-triglycerides showed no significant change during the treatment (Fig 7).

FSH and LH decreased ($P < 0.001$) during the treatment (Fig 8) and this decrease was more pronounced than that following treatment with Trisekvens®. LPE increased ($P < 0.001$) to values corresponding to those found during the secretory phase of the menstrual cycle (Fig 9).

Group III Estrofem® forte

There is a statistically significant ($P < 0.001$) decrease in S-cholesterol levels after 12 months of treatment (Fig 10). As in group II cholesterol values in patients with elevated levels prior to treatment decreased to normal after 6 months of treatment (Fig 7).

There were no significant changes in S-triglycerides (Fig 11).

FSH and LH decreased ($P < 0.001$ and $P < 0.01$ respectively) during treatment (Fig 12). LPE increased to values corresponding to those found

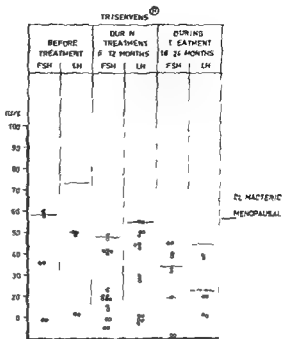


Fig 4 Serum levels of FSH and LH following treatment with Trisekvens®

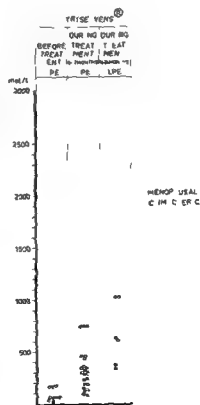


Fig 5 Serum levels of LPE following treatment with Trisekvens®

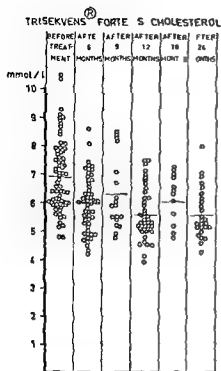


Fig 6 S Cholesterol levels following treatment with Trisekvens[®] forte

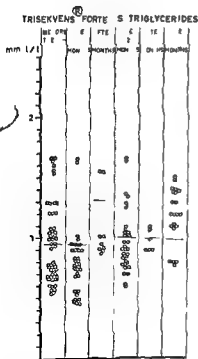


Fig 7 S Triglyceride levels following treatment with Trisekvens[®] forte

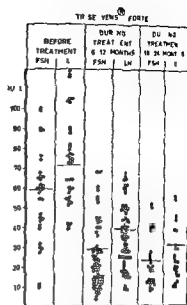


Fig 8 Serum levels of FSH and LH following treatment with Trisekvens[®] forte

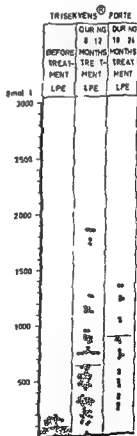


Fig 9 Serum levels of LPE following treatment with Trisekvens[®] forte

ESTROFEM® FORTE & CHOLESTEROL

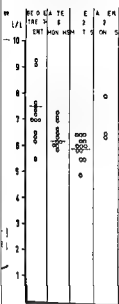


Fig 10 S-Cholesterol levels following treatment with Estrofem® forte

ESTROFEM® FORTE & TRIGLYCERIDES

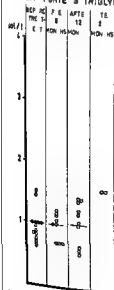


Fig 11 S-Triglyceride levels following treatment with Estrofem® forte

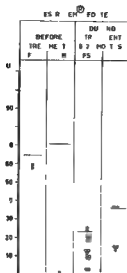


Fig 12 Serum levels of FSH and LH following treatment with Estrofem® forte

ESTROFEM® FORTE

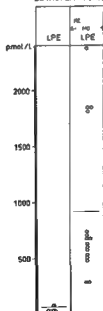


Fig 13 Serum levels of LPE following treatment with Estrofem® forte

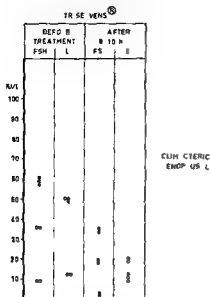


Fig 14 Serum levels of FSH and LH 8-10 hours after intake of one single tablet of Trisekvens®

during the luteal phase of a normal menstrual cycle (Fig 13)

We noticed that the hormone values during the treatment were very variable. 42 patients therefore underwent a controlled investigation. They were instructed to take a hormone tablet which contained only oestrogens 8 hours before the hormone determination. The results are listed in Figs 14-17. It is noted that the values still differ considerably from each other which may depend upon large individual differences in absorption.

Influence upon Climacteric Symptoms

Treatment of patients having irregular menstruation with trisekvens® resulted in a regulation of their cycle.

The vasomotor symptoms disappeared after a few days medication. The patients were able to sleep better than before and in many cases stopped their intake of hypnotic drugs.

Using psychological tests Fedor Freybergh (7) has demonstrated that treatment of climacteric and postmenopausal women with 17 β oestradiol valerate (Progynon®) had a positive effect upon depression, performance, concentration and memory disturbances. No systematic psychological tests have been applied in the present study but most patients claimed positive effects upon depression, nervousness, performance, memory and concentration.

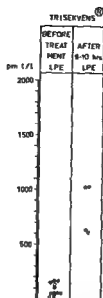


Fig 15 Serum levels of LPE 8-10 hours after intake of one single tablet of Trisekvens®

Influence upon Symptoms of Oestrogen Deficiency

In all cases atrophy of the vulva and of the vaginal mucosa ceased following oestrogen treatment. A positive effect was also found on senile vaginitis. Patients who had been suffering from recurrent cystitis and urethritis improved considerably during treatment with trisekvens® forte and estrifol forte. Positive effects were also noted upon the skin.

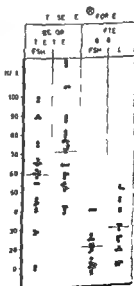
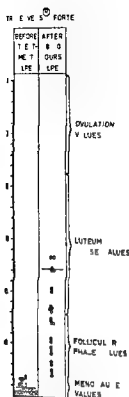


Fig 16 Serum levels of FSH and LH 8-10 hours after intake of one single tablet of Trisekvens®



17 Serum levels of LPE 8-10 hours after intake of a single tablet of Trisekvens® forte

swollen and sore finger joints frequently seen in menopausal women improved considerably during treatment with trisekvens® forte and estrofem® forte

Together with Dahlen Jacobson & Lamke (3) we studied the effect of trisekvens® forte and estrofem® forte on the physiological bone loss in 13 patients receiving oestrogen and in 13 controls. It was found that the skeletal mineral deposits increased significantly in the hormone treated group with an average of 6% during a one year follow up period. There was no correlation between bone mineral change and the age of the patient.

As previously shown cholesterol values decreased during oestrogen treatment while the levels of triglycerides remained unchanged.

Side Effects

Practically no side effects were observed in the 145 patients from which two or more serum samples were obtained. No nausea and vomiting was observed. Mastodynia appeared in 12% of the pa-

tients at the beginning of the treatment but disappeared after 2 months. Irregular uterine bleeding due to irregular tablet intake occurred in 6 patients. A curettage was performed in all these cases and revealed no malignancy. 3 patients using trisekvens® forte suffered from copious withdrawal bleeding and the medication was changed to estrofem® forte for 28 days followed by a tablet free week. Four patients suffered from premenstrual and menstrual pain during treatment with trisekvens® forte and these patients also changed to estrofem® forte for 28 days followed by one tablet free week.

Of the remaining 64 patients receiving the NOVO tablets and from whom we obtained less than two serum samples 34 stopped the medication for the following reasons. Fourteen women changed to another type of oestrogen. One of them wanted to have injections instead of taking pills. 7 women went to other doctors who prescribed conjugated oestrogens (Promant®) or 17 β -oestradiol valerate (Progynon®) instead of trisekvens®. 2 women developed oedema and 4 complained of copious withdrawal bleeding. In these cases 2 mg of 17 β -oestradiol valerate (Progynon®) daily was prescribed.

Twenty patients completely stopped their hormone medication. Two patients in this group had hypertension (190/100 and 160/90 respectively). Hereditary factors might have been involved as their parents had also been hypertensive. They went to their general physician who declared that oestrogen medication was dangerous for them and they therefore stopped the medication. One patient did not like to take tablets every day. 2 became oedematous and gained weight. One suffered from nausea. 2 did not like to menstruate and one patient stopped because she read in a newspaper that oestrogens could provoke cancer. One patient who suffered from depression did not improve during treatment with trisekvens® forte and 8 patients did not return for re-examination after one year.

Two patients developed a mammary carcinoma which was found during the check up. One was a 52 year-old woman who had last menstruated 6 months previously and was treated cyclically with conjugated oestrogens (Promant®) and 15 mg of allyloestrenol (Gestany®). One year later a lump was found in her breast and biopsy revealed a medium-well differentiated macrocytic ductal cancer. She received immediate surgical treatment.

The other cancer case was a 57 year old woman who had been treated with trisekvens® forte for 15 years. Her cancer was small and without metastases and no postoperative treatment was carried out. Both patients are now doing well. According to Kupperman (15) the expected frequency of breast cancer in the age group 50-60 is 5 cases per 100 women. The present study include 2 cases in 200 oestrogen treated women. During the same period of time we found 3 cases of breast cancer among the patients who had not been treated with oestrogens.

DISCUSSION

The treatment of climacteric and postmenopausal women with trisekvens®, trisekvens® forte and estrofem® forte considerably improved their general condition and relieved their climacteric symptoms. Few side effects were observed. Fibromas have been reported to increase in size during oestrogen treatment although this occurs very rarely. Patients with small fibromas were included in the present study but in no case was growth of the tumour observed. It is also worth mentioning that no changes in the blood electrolytes or liver enzymes were observed during the treatment.

According to Lauritzen (1973) the dose of oestrogen used in replacement therapy should always be individualized according to the need of the patient and the lowest maintenance dose should be administered. From a practical point of view however such individualized doses are difficult to determine.

The patients taking trisekvens® showed higher FSH and LH levels during the treatment than those taking trisekvens® forte or estrofem® forte. Furthermore patients taking trisekvens® showed a tendency to higher blood levels of triglycerides after 2 years of intake. The dose of oestrogen in trisekvens® may be insufficient to prevent the rise in blood triglyceride levels which develops after the menopause and it also exerts a less pronounced suppressive effect upon the hypothalamic-pituitary axis especially in menopausal women. The mean levels for serum LPE in menopausal subjects during trisekvens® treatment corresponded to those found in the proliferative phase of a normal menstrual cycle.

Treatment with trisekvens® forte and estrofem® forte resulted in a more pronounced suppression of the FSH and LH levels. Serum triglycerides re-

mained unchanged during this treatment, but cholesterol levels were significantly decreased. High cholesterol levels fell to normal values after treatment with trisekvens® forte and estrofem® forte as well as with trisekvens®. The serum LPE levels in menopausal subjects during treatment with trisekvens® forte and estrofem® forte corresponded to those found in the luteal phase of a normal menstrual cycle.

Trisekvens® forte and estrofem® forte seem to be sufficient for the prevention of the atrophic changes associated with postmenopausal oestrogen deficiency while trisekvens® in some cases was ineffective in this respect. Referring to the development of osteoporosis only trisekvens® forte and estrofem® forte were tested and treatment with these preparations resulted in a significant increase in the bone mineral reserve.

It can be concluded from the above discussion that oestrogens for replacement therapy should be given in doses which give rise to serum LPE levels corresponding to those found during the normal luteal phase.

The patients should be re-examined at least once a year. In our series the patients were given 3 tablets for a 3 months period and thereafter had to report as to their general health and any side effects before receiving a new 3 month supply.

After the menopause oestrogens are still produced for some time and many women have climacteric symptoms. Women without symptoms often want to be treated with oestrogens because they have read about the advantages of such therapy. There is sufficient evidence concerning the benefits of oestrogen therapy to allow treatment of patients without climacteric symptoms.

On the other hand many patients have a negative attitude to oestrogen treatment partly due to articles in newspapers and magazines dealing with the risk of cancer. In this series there were few women who discontinued their oestrogen medication who never returned for new tablets. This may be depended upon the fact that the patients were informed before treatment commenced. Some patients prefer to have climacteric symptoms and want to be ill to play the role of a woman. Some patients also have an irrational fear that by not taking oestrogen therapy one is tampering with the natural nature. Other women feel a sense of relief when menstruation ends. They have never been so at being a woman and now at last they are.

the detestable evidence of their womanhood. Pa-
 ts with a negative attitude to oestrogen medica-
 tion should not be persuaded to alter their ideas

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THE EFFECT OF VARIOUS DOSES OF ORAL OESTRADIOLVALERATE AND OESTRIOLSUCCINATE ON URINE CALCIUM/CREATININE SERUM FSH AND ENDOMETRIUM IN CASTRATED WOMEN

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Abstract: The effects of various doses recommended for oral use of oestradiolvalerate and oestrilsuccinate on urine calcium/creatinine, serum FSH and endometrium were investigated in two castrated women. Oestrogen treatments were given in 6-week periods separated by 2 weeks without treatment. After various oestrogen treatments, serum FSH generally decreased clearly and urine calcium/creatinine slightly. The proliferative effect on endometrium was investigated histologically. In no period was hyperplastic endometrium found.

The size of the dose in oestrogen substitution therapy is often determined by the effect on vegetative symptoms, yet most of these symptoms are not good indicators of the therapy's effectiveness. According to Launzen, the only specific climacteric complaints are hot flushes combined with dizziness and sweating and a paroxysmal rise in blood pressure (4). The main purpose of long-term oestrogen therapy is to prevent or postpone degenerative postmenopausal tissue changes such as osteoporosis and atrophy of skin and mucous membranes. No correlation has been found between vegetative symptoms and cyto-hormonal status or skin changes (2, 7). One of the most common side effects associated with oestrogen therapy is excessive endometrial proliferation and uterine bleeding. It has been suggested that the increased incidence of endometrial carcinoma (9) may have to do with the greatly increased use of oestrogens. Apart from these effects on postmenopausal tissue changes, it is important to ascertain the effect of various oestrogen doses on the endometrium. The purpose of the study was to determine the effect of various doses of oestradiolvalerate and oestrilsuccinate on urinary calcium/creatinine, serum FSH and endometrium.

SUBJECTS AND METHODS

The subjects were 2 female patients, 31 and 35 years of age. Both had undergone bilateral oophorectomy 17 years previously for ovarian tumours but the uterus had been preserved. The patients had last received oestrogen treatment about one month before the study started. In the present investigation, oestrogen therapy was given in 6-week periods separated by 2 weeks without treatment. At the beginning and end of each treatment period, the urinary calcium and calcium/creatinine and serum FSH were measured and an endometrial biopsy specimen was taken from the lateral wall of the uterine body.

Urine samples were collected after an overnight fast from 8 p.m. the previous evening. The urine was collected between 8 and 10 a.m. the overnight urine having been discarded. Calcium and creatinine were estimated in urine samples by standard auto-analyzer techniques (1). Serum FSH was determined radioimmunologically (5).

RESULTS

Urine calcium and calcium/creatinine

The oestrogen doses used daily in the 6-week treatment periods and the effect of treatment on urine calcium and calcium/creatinine are presented in Figs 1 and 2.

FSH

The effect of the same oestrogen treatments on serum FSH is shown in Fig. 3.

Effect on the endometrium

The effect of the 6-week oestrilsuccinate therapy (2 mg/day) on the endometrium was minimal. Mild signs were noticed in the glandular epithelium.

The corresponding treatment with oestradiolvalerate (1 mg/day) or with oestradiolvalerate 1 mg + oestrilsuccinate 2 mg/day caused weak pro-

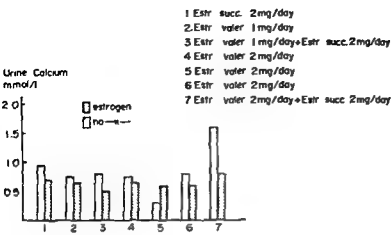


Fig 1 Urine calcium before and after 6 weeks of oestrogen therapy

liferation The endometrium was low and there was mild stromal proliferation A more pronounced effect of oestrogen therapy was seen with oestradiolvalerate 2 mg/day Proliferation was observed in the surface epithelium glands and stroma The 6-week treatment has been given three times separated by 2 week intervals without treatment After the 2 week interval there were mild signs from the effect of oestrogen At the end of the third treatment period the endometrial proliferation was about the same as at the end of the two previous treatment periods A fairly marked proliferation was observed when oestradiolvalerate 2 mg + oestradiolsuccinate 2 mg/day were used for 6 weeks (Fig 4)

DISCUSSION

Plasma and urinary calcium are mildly elevated in postmenopausal women (8) The calcium returns to the premenopausal level with oestrogen therapy Gallagher & Nordin consider that determination of the fasting urinary calcium/creatinine in addition to the urinary hydroxyproline/creatinine gives a better index of a reduction in bone resorption and they regard these measurements as most useful when assessing the effect of oestrogen treatment on bone In this study the 6 weeks of oestrogen treatment with various doses generally led to effect was seen with oestradiolsuccinate 2 mg/day After a 2 week interval there was always a slight increase in urinary calcium and calcium/creatinine The

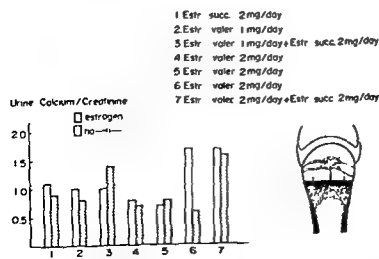


Fig 2 Urine calcium/creatinine before and after six weeks of oestrogen therapy

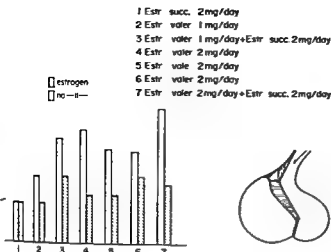


Fig 3 Serum FSH before and after six weeks of oestrogen-therapy (means of the values of two women)

hism by which oestrogen affects the skeleton is not entirely clear but its inhibiting effect on bone resorption can be regarded as a blockage of the action of parathyroid hormone on bone (1). Oestrol has been considered to have a specific effect on vaginal and cervical epithelium (6). In this study 6 weeks of oestrogen in the glandular epithelium of the endometrium. In Heuser and Kemmler's study (3) 13–34 days of oestradiol succinate therapy (2 mg/day) caused endometrial proliferation in 9 out of 11 postmenopausal women. The most pronounced effect on the endometrium was observed in patients whose last menstrual period had occurred less than one year previously. Furthermore daily treatment with 4–8 mg caused generalized endometrial proliferation. It is thus evi-

dent that oestrol also affects the endometrium. The question concerns the dosage. The 6-week treatment with oestradiolvalerate (2 mg/day) was given three times with 2 week intervals without treatment. No cumulative effect of oestrogen was observed. At the end of the third treatment period the proliferation was about the same as at the end of the two previous treatment periods and corresponded to the middle of the proliferative phase of the normal menstrual cycle. A possible factor in the etiology of endometrial carcinoma is thought to be cystic glandular hyperplasia and precancerous adenomatous hyperplasia. In this study no changes consistent with hyperplasia were seen. No uterine bleedings occurred during or after oestrogen treatments.

Endometrium after six weeks of oestrogen-therapy

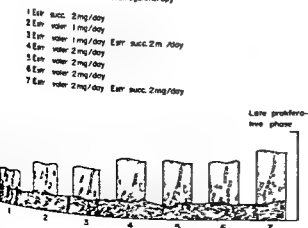


Fig 4 Endometrial thickness after six weeks of oestrogen-therapy. Before treatment endometrium was quite atrophic (The evaluation is based on histologic examination)

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CHANGES IN SERUM LIPIDS DURING TREATMENT WITH NORGESTREL OESTRADIOL VALERATE AND CYCLOPROGYNON®

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Abstract The serum concentrations of triglycerides, esterol and free glycerol were determined in 23 astenic women before and after the administration of 3 different steroid drugs. Each drug was given within a period of 12 weeks (3 cycles). Period I Norgestrel 0.5 mg daily from the 17th to 21st day of each cycle. Period II Oestradiol valerate (Progynon®) 2 mg daily from the 2nd to 11th day of each cycle. Period III Oestradiol valerate 2 mg + 0.5 mg *dl* norgestrel from day 12 to 21 of each cycle (Cycloprogyon®). A significant decrease in triglycerides was observed following the administration of Norgestrel and Cycloprogyon®, whereas oestradiol valerate had no effect on the triglyceride levels. On the other hand oestradiol valerate following a period of norgestrel produced an increase in serum cholesterol levels.

A significant decrease in the serum concentration of triglycerides has previously been demonstrated in astenic women treated with Cycloprogyon® (2 mg oestradiol valerate daily from the 2nd to 11th day followed by 2 mg oestradiol valerate + 0.5 mg norgestrel daily from the 12th to 21st day of the cycle) (3). This was accompanied by a non significant decrease in serum cholesterol, free cholesterol and free fatty acids.

The present investigation was carried out in order to determine the drug responsible for this decrease in serum lipids, i.e. norgestrel, oestradiol valerate or the sequential combination of the above two.

MATERIAL AND METHODS

The material consisted of 23 women between the ages of 40 and 55 years, average 45 years (S.D. ± 4.7). They had all been referred to the Gynaecological Department of the

Odense University Hospital owing to menometrorrhagia. Diagnostic curettage was carried out in order to exclude the possibility of malignancy. In addition to the above symptoms 18 of the patients complained of vasomotoric and/or other climacteric symptoms. A clinical examination revealed no other pathological conditions.

The set up of the investigation was as follows. No hormonal therapy for at least 3 months prior to being admitted to the trial. Two clinical examinations (including the withdrawal of blood samples) with an interval of two weeks. Thereafter each patient was given firstly norgestrel for three cycles, secondly Progynon® for three cycles and thirdly Cycloprogyon® for three cycles. Each cycle was 28 days.

The hormones administered orally were as follows: Norgestrel 0.5 mg *dl* norgestrel daily from the 17th to 21st day of the cycle. Progynon® 2 mg oestradiol valerate from the 2nd to 21st day of the cycle. Cycloprogyon® 2 mg oestradiol valerate daily from the 1st to 11th day and 2 mg oestradiol valerate + 0.5 mg *dl* norgestrel daily from the 12th to 21st day of the cycle.

Each patient was examined between 8 and 9 a.m. following a minimum period of 8 hours of fasting. All the examinations, apart from those carried out prior to being admitted to the trial, were performed on the 27th day of the cycle, i.e. on the morning after the patient had taken the last tablet of each treatment cycle. The patients were weighed, the blood pressure measured and venous blood samples withdrawn for the determination of serum triglycerides, total serum cholesterol and serum free glycerol.

The triglycerides and free glycerol were determined according to the method of Eggstein & Kreutz (8) and the cholesterol according to the method of Huang et al. (9).

No attempt was made in the present investigation to evaluate the symptomatic effect of the treatment.

Wilcoxon's test for paired comparison has been employed for the statistical evaluation of the hormonal effect on the serum lipid concentrations. In this manner each patient acted as her own control. The limit of significance employed was $p < 0.05$.

Table 1 Mean values (\pm S D) of serum triglycerides, cholesterol and free glycerol in 31 women before and after 3 sequential periods of hormonal treatment each consisting of oestradiol valerate and a combination of these (Cycloprogynon®) respectively

	Pre treatment	Norgestrel			Oestradiol valerate			Oestradiol valerate + norgestrel		
		I	II	III	I	II	III	I	II	III
Triglyceride mM/l	1.17	0.87*	0.94	0.87*	1.00	1.10	1.18	0.96	0.91	0.88
S D	0.29	0.32	0.25	0.20	0.77	0.31	0.29	0.30	0.31	0.28
Cholesterol mM/l	6.4	6.0	6.3	6.4	6.7	6.9	7.0	6.3	6.1	6.3
S D	1.7	1.0	1.2	1.3	1.1	1.1	1.4	1.3	1.0	1.1
Glycerol mM/l	0.062	0.060	0.063	0.076	0.074	0.066	0.061	0.060	0.061	0.063
S D	0.020	0.039	0.040	0.050	0.055	0.035	0.073	0.048	0.047	0.045

*= $p < 0.01$ **= $p < 0.05$

RESULTS

The serum concentrations of triglycerides, total cholesterol and free glycerol are shown in Table 1. In this the mean values and standard deviations for the whole material during the pre-treatment phase are compared with the results obtained in the three cycles of each of the three treatment periods.

The triglyceride values were significantly lower ($p < 0.01$) during treatment with norgestrel in all three cycles. These gradually returned to pre-treatment levels during the administration of progynon®. During treatment with Cycloprogynon® the triglyceride levels again decreased significantly in all three cycles ($p < 0.05$). The values during norgestrel and Cycloprogynon® periods were not mutually statistically different.

The total cholesterol concentrations were found to be significantly higher during treatment with progynon® ($p < 0.05$) as compared to the pre-treatment, norgestrel and Cycloprogynon® periods. The cholesterol values were almost similar during these three periods.

No significant alterations were observed in the concentrations of free glycerol throughout the whole investigation. Neither were changes seen in the weight (± 2 kg) nor in the blood pressure.

DISCUSSION

The effect of the Pill on lipid metabolism has been the object of discussion and investigation for quite a number of years. Thus even within the last three years four investigations have been published (4, 8, 10, 19) together with a survey (1) on the effect of combined tablets containing synthetic

oestrogens and gestagens used for contraceptive purposes. Beck (1) summarizes the effect of contraceptive steroids on lipid concentrations in normal women as follows: "The administration of synthetic oestrogens increase the triglyceride and nortestosterone derivatives counteract the effect. Both effects seem to be dose related. The changes in serum cholesterol concentrations appear to be related to the amount of gestagen in conjunction with a synthetic oestrogen."

These studies and most of the discussion have been confined to the effects of synthetic oestrogens on the lipids of fertile women, while little attention has been paid to women in the menopausal phase of life. The present investigation was carried out on such women in order to ascertain the effect of genuine oestrogen, oestradiol valerate and norgestrel both alone and in combination on the lipid levels.

Some authors have observed that the effect of genuine oestrogens on the lipid metabolism differs from that of the synthetic oestrogens. Thus Leden & Borggaard (12) found that a pill containing 2 mg of 17β oestradiol + 1 mg oestrol + 1 mg norethisterone given to healthy women between the ages of 21 and 30 years produced no effect on the serum triglycerides or serum phospholipids, whereas a pill containing 0.05 mg of oestradiol + 1 mg norethisterone given to the same women raised the serum lipid levels. On the other hand in a later investigation (13) they found that 2 mg oestradiol + 1 mg oestrol or a tablet containing twice this dosage brought about a decrease in triglycerides, cholesterol and phospholipids when given to post-menopausal women further lowering

stri* and ethinyl oestradiol raised the level of lipids Borglin & Staland (2) obtained the results but Launitzen (11) using oestradiol alone was unable to see any effect on cholesterol or triglyceride concentration. Two investigators (15-18) found that oestradiol alone lowered the cholesterol levels but not of the triglycerides. Subcutaneous oestradiol (25 mg) was observed to decrease the triglycerides but have no effect on the cholesterol (3).

There are many reasons why conflicting results obtained from similar clinical trials. For example there can be differences in the patient's diet, the mode of administration of the drug, a predisposition to diabetes (14), the age of the patient in relation to the menopause and a number of others.

On the whole it appears that most genuine oestrogens lower the serum lipid levels. Further, synthetic oestrogens raise lipid concentrations, however the latter is well-documented in contrast to the former. The genuine oestrogen oestradiol valerate has been employed in the present investigation and the results show that it has no effect on triglyceride values (Table I) but that it does lower the level of cholesterol ($p < 0.05$) when given alone. However, when given together with norgestrel (Cycloprogynon*) it had no influence on cholesterol concentrations. This increase in serum cholesterol values after the administration of oestradiol valerate has not been reported earlier.

We found that norgestrel both alone and when given together with oestradiol valerate brought about a significant decrease in the triglyceride concentrations and that these values returned to pre-treatment levels at mid-period where oestradiol valerate was the only drug administered. Investigations into the effect of *dl* norgestrel have given somewhat conflicting results (6, 7, 17). In two studies the cholesterol levels decreased (6, 7) while triglyceride concentrations fell in two others (6, 17) although this was only significant in one (17). However, it has been shown that 0.075 mg of norgestrel caused a more pronounced decrease in triglyceride levels than 0.050 mg (6). These findings regarding the triglyceride levels are in agreement with the results obtained in the present study where 0.050 mg of *dl* norgestrel once a day for ten days produced a significant fall in triglycerides. Schneider et al. (16) have published the results of an investigation on somewhat similar lines to those of

our study. They administered the same sequential drug Cycloprogynon* to 20 post-menopausal women with an average age of 52 years. They state that there is no significant reduction in serum triglyceride concentrations during treatment cycles as compared to pre-treatment and placebo cycles. However, a closer scrutiny of their reported values shows that the mean values at the end of the therapy cycles are approximately 30% lower than the mean values obtained in mid-cycle at which time the patients had only received oestradiol valerate.

Briggs (4) has shown that norgestrel counteracts the influence that many synthetic oestrogens have on serum lipids.

In conclusion it may be stated that the norgestrel component of the sequential drug Cycloprogynon* is responsible for the decrease in serum triglycerides in climacteric women. Further, that oestradiol valerate has no significant influence on the triglyceride levels but that it increases the concentration of cholesterol.

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COAGULATIVE AND FIBRINOLYTIC STUDIES ON POSTMENOPAUSAL WOMEN TREATED WITH A NEW NON STEROIDAL OESTROGEN

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Abstract Treatment of postmenopausal women with a nonsteroidal oestrogen (P1496) in a dose of 50 mg a day 14 days before operation for prolapse did not suppress histochemically determined fibrinolytic activator concentration of the vessel wall. Neither was any change found in concentration of P&P-complex (prothrombin factor X) factor VIII antithrombin III α_1 antiplasmin α_2 -macroglobulin or the inhibitors of urokinase activated plasminogen activation. Nothing suggested a thrombogenic effect of this non steroidal oestrogenic compound.

Changes in the vessel walls have long been thought to play a role in the pathogenesis of thrombosis. Åstedt (23) showed that the endothelium of certain vessels contain activators of the fibrinolytic system. Svanberg (13) found the release of fibrinolytic activators from the vessel wall during venous occlusion to be deficient and/or the fibrinolytic activator content of the vein walls to be low in 73% of patients with recurrent idiopathic thrombosis. The same defect in the fibrinolytic system has also been found in women who had thrombosis during use of oral contraceptives and who were free of symptoms and signs of disease when examined (26). The oestrogenic component in combined oral contraceptives (12) and oestrogens alone (2, 4, 14) have been found to increase the frequency of thrombotic complications. Åstedt (24) found ethinylloestradiol to lower the fibrinolytic activity of vessel wall but Åstedt & Jeppsson (25) found such decrease following treatment with 17 β oestradiol and they concluded that 17 β oestradiol may turn out to be safer than synthetic oestrogens. Postmenopausal substitution therapy. Kullander & Svanberg (15) studied a new non steroidal

oestrogenic preparation P1496 (Sandoz) and found this drug to be effective in the treatment of climacteric symptoms.

This paper concerns the fibrinolytic activity in biopsy specimens of superficial veins obtained from postmenopausal women before and after treatment with this non steroidal oestrogen (P 1496) as well as determination of some coagulation factors antithrombin III and components of the fibrinolytic system.

MATERIAL AND METHODS

The clinical material consisted of 10 healthy women aged 45 to 70 (mean 63.4 years) who were to undergo operation for uterine prolapse. They were all given P1496 in a dose of 50 mg a day for three weeks before operation and blood samples for determination of P&P factor VIII antithrombin III α_1 antitrypsin α_2 macroglobulin and inhibitors of urokinase induced plasminogen activation (urokinase inhibitors) and vein biopsy specimens were obtained before and on the last day of treatment.

Evidence of an oestrogenic response in the endometrium on the last day of treatment which was immediately followed by the operation (including curettage) was regarded as proof that the patient had taken the drug.

Coagulation and fibrinolytic studies

Prothrombin + factor VII + factor X (Owren's P&P test) (19) Normal range in plasma 80-170°

Factor VII (AHF) (17) Normal range in plasma 60-160°

Antithrombin III Immunochemical method according to Fagerhol & Abildgaard (6) Normal range in serum 60-140°

α_1 -antitrypsin Immunochemically determined based on the rocket method by Laurell (16) Normal range in serum 80-120°

α_2 -macroglobulin Esterolytic method (9) Normal range in serum 80-170°

Table I *Fibrinolytic activity of hand veins before and at end of treatment with a non steroidal oestrogen (P 1496)*

The fibrinolytic activity was histochemically determined and is expressed in arbitrary units (see text)

	Range	Median value
Before treatment	5-9	7.5
End of treatment	5-10	7.5

Inhibitors of plasminogen activation by urokinase (urokinase inhibitors). Clot method (21) Normal range in serum 60-140%

Fibrinolytic activity in the vein vessel wall The principle of the method (23-26) was as follows. Cryostat sections of the specimens collected on glass slides were covered with a plasminogen rich fibrin layer. After certain periods of incubation the slides were fixed and stained. If plasminogen activators were present they converted plasminogen into plasmin with digestion of the overlying fibrin layer. They varied in size with the activator activity.

Vein biopsy specimens were taken—with the consent of the informed patients—from the dorsal side of the hand under local anaesthesia. After a longitudinal incision of the skin a 0.5 to 1.0 cm segment of the vein was exposed carefully dissected and excised.

The specimens were quickly frozen in rapidly expanding CO₂ to prevent freezing artefacts. They were packed hermetically in Parafilm or metallic foil to prevent drying and stored at -60°C.

The vein biopsy specimens were cut on an International Harris cryostat in sections (8 µm) which were placed on pre-cleaned glass slides. Four slides were prepared for each sample. The sections on each slide were covered with 0.06 ml of bovine fibrinogen prepared essentially according to Brakman's modification (3) of Astrup & Mullertz (1) double ammonium sulphate precipitation method in a concentration of 1% in phosphate buffer (pH 7.8 ionic strength 0.15) and of 10 µl thrombin (Topo-Plasmin 20 NIH units/ml unbuffered saline). The fibrinogen-thrombin mixture was spread over an area of 10 cm² to obtain a fibrin film about 0.07 mm thick. To stabilize the fibrin film the slides were left at room temperature (21 to 24°C) in a moist chamber for 30 min. One of the slides was immediately fixed in formalin while the remaining three slides were transferred to another moist chamber at 37°C and incubated for 10, 20, 30 min respectively after which they were fixed in formalin. The slides were stained with Giemsa solution and Harris haematoxylin. Fibrinolysis was reflected by clear lytic areas in the fibrin film at the site of fibrinolytically active cells. Three fairly distinct grades of fibrin digestion were recognized. Grade I signified microscopical punctate areas in most of the sections. Grade II meant gross lytic areas of irregular outline and sometimes confluent and Grade III meant dissolution of most or all the fibrin in contact with the sections.

A Grade I slide was allotted 1 point, a Grade II slide 2 points, a Grade III slide 3 points. The total number of

points scored by the set of four slides was taken as a measure of the fibrinolytic activity of the veins. The normal range was 6.0 to 10.0 arbitrary units, the average value being 7.5.

Statistical methods The group were compared by use of a Student's *t*-test. The difference in the fibrinolytic activity of the veins was analysed with Wilcoxon's sign-sum test (5).

RESULTS

A high degree of oestrogenic influence in the endometrium was found in the endometrium of all the patients treated. The fibrinolytic activity of the venous specimens was normal and remained unchanged during treatment (Table I). Neither were any changes found in the concentration of macroglobulin or urokinase inhibitors. The concentration of α_2 -antitrypsin was significantly increased after treatment though still within normal range. The values found for the factors—P&P complex, factor VIII and prothrombin III—were all normal and no changes were found after treatment (Table II).

DISCUSSION

Many authors (22-30) have found that the use of a synthetic oestrogenic preparation such as mestranol is followed by an increase in fibrinogen and a slight increase in factor VIII. They have also reported an increase in fibrinogen following administration of conjugated equine oestrogen in large doses. But Åstedt & Jeppsson (25) have found that the natural hormone 17 β -oestradiol had no such effect on the fibrinogen levels.

Howie et al. (10) found the synthetic oestrogen

Table II *Coagulation factors, antithrombin and components of the fibrinolytic system before and at the end of the treatment with a non steroidal oestrogen (P 1496)*

	Before treatment (Mean \pm S.D.)	End of treatment (Mean \pm S.D.)
P&P	105 \pm 14	101 \pm 14
Factor VIII	24 \pm 9.3	14.9 \pm 4.4
Antithrombin III	105 \pm 17	96 \pm 8
α_2 -antitrypsin	96 \pm 9.8	111 \pm 8.8
α_2 -macroglobulin	114 \pm 13.5	112 \pm 11
Urokinase inhibitors	111 \pm 23.1	111 \pm 23.1

$p < 0.001$

variation mestranol to lower the antithrombin III activity and Fagerhol et al (7) showed that combined oestrogen progesteron oral contraceptives lowered the antithrombin III level by about 15%. Åstedt & Jeppsson (25) however found no significant decrease in antithrombin III during the use of 17β oestradiol.

In the present study we did not notice any change in the coagulation factors (P&P-complex factor, antithrombin III) during treatment with P 1496 which in this respect behaves like a natural oestrogen.

An increase in the α_2 macroglobulin has been reported by Howie et al (10) during the use of mestranol but no such increase has been found in connection with the use of ethinyloestradiol (24) or 17β oestradiol (25). In the present study no change was found in α_2 macroglobulin or urokinase inhibitors during the treatment with P 1496. However, antitrypsin rose slightly from a mean of 96.2% to 119% but was still well below the normal limit (170%).

The normal content of fibrinolytic activators in the vessel walls and a normal ability to release these activators to the blood stream seems to be the most important factors for the fibrinolytic defence system against thrombosis. As mentioned Åstedt (24) found a decrease in the content of fibrinolytic activators in menopausal women receiving ethinyloestradiol in a dose of 0.25 mg a day for 10 days. In contrast no such decrease was found by Åstedt & Jeppsson (25) in women taking 10 mg of 17β oestradiol a day for 10 days. As far as we know there are no reports of increased frequency of thrombosis following treatment with naturally occurring oestrogens.

In the present study a dose of 50 mg P 1496 a day was comparatively lower but given for 21 days did not result in any decrease in the fibrinolytic activators in the vessel walls (Table I). The investigation thus showed no evidence of a thrombogenic effect of P 1496, a drug found useful in combating climacteric symptoms (15).

As the number of climacteric and postmenopausal women given oestrogenic therapy will presumably rapidly increase, this substitution gives them relief as stated by Furuholm (8) it is of great importance that not only the beneficial effect but also eventual side-effects be carefully investigated and charted. Besides the risk of tumours and endometrial carcinoma, mammary carcinoma-

ma—that of thromboembolism seems to be the most important. The difficulties in such investigations are however considerable. The natural frequency of the diseases mentioned as side effects is obscure and the possibility of fluctuation owing to variation of risk factors—other than the administration of oestrogenic drugs—should warn against too hasty conclusions (pointed out for endometrial carcinoma by Ingelman Sundberg (11)).

Coagulative and fibrinolytic studies with available methods to test thrombogenic properties is one way of elucidating the thrombogenic risks inherent in oestrogenic drugs. Comparison of oestrogens of different kinds, natural and synthetic, with and without the steroid nucleus, to find the most effective and least risky is of both theoretical and clinical interest.

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SOME METHODOLOGICAL ASPECTS IN THE PSYCHOSOMATIC GYNAECOLOGY

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Psychosomatic medicine—and thus psychosomatic gynaecology—is presented as an approach to be used by doctors when meeting the patient. At the same time it is a method, a technique, trying to elucidate the mechanisms in the most complicated interplay between psychological, somatic and social parts of the whole. Some diagnostic methods which can be used in this connection are discussed. The basic aim of the paper is to show the necessity and the possibility for the gynaecologist to use new techniques from the psychodiagnostic field in his daily practice in order to achieve a better understanding of his patient's complaints and problems.

The psychosomatic approach is equal to good medicine (J. Apley)

Psychosomatic medicine—and thus psychosomatic gynaecology—cannot be considered as a separate medical speciality. It is much more an approach to be used by doctors, psychologists etc. when meeting the patient. It is based on the conviction that no patient is disturbed exclusively in body or in mind, but it is always the whole person who is ill, and otherwise the whole person is healthy. If this be the case, we can speak neither about psychosomatic diseases nor about disorders, but only about the psychosomatic approach.

As Apley (?) pointed out, the word psychosomatic is a heritage of Cartesian duality, with its intellectual supermarket unfortunately it strengthens the idea that the mind and body are separate.

We know both from physiology and psychology that in healthy people all bodily processes and functions are associated with psychological ones, especially emotions—and vice versa. This also applies to pathophysiology and psychopathology

concerning people when ill. Pathological changes in the body will be associated with sick emotions and again vice versa. This is a highly mechanical view of one speaking about psychosomatic illnesses, understanding only ulcus duodeni, asthma bronchiale etc. In these diseases most studies have demonstrated the connection between emotions and organic changes. The lack of studies in other diseases does not prove the converse. Following the classical dialectic way of thinking, we must come to the only acceptable conclusion that there is no possibility of separating bodily from psychological phenomena and that the psychological factors are important in all health and in all disease.

In this paper we must nevertheless, for semantic reasons, use the pragmatic scientific terminology which sometimes seemingly will contradict our intentions. The only person to solve this personal dilemma is the reader himself through his interpretations.

How much then the psychological factors will influence the particular symptom syndrome disturbance or disease will depend on the patient's personality structure on his reaction patterns on the special circumstances at the onset of the disease on the patient's frustration threshold and on psychophysical maturity and state of development (7). Nevertheless the whole clinical picture will be influenced by the social factors and the whole life situation of the patient.

Jores (12) speaks in this connection of human disorders—a group of disorders in which not so much physical injury but rather the patient's problems with life and his human failings are expressed.

When trying to explain the tentative mechanisms

underlying the psychological influences on bodily changes we can quote a psychosomatic model of Luban Plozza & Poldinger (17). According to this model certain emotions give rise to certain autonomic changes. Via the diencephalon and the autonomic nervous system neurotic and unconscious factors exert an effect on the body which may result in functional impairment and damage to the organs.

According to the neurophysiologists all stress situations lead to an activation of the hypothalamus which immediately sets protection and defence mechanisms in train along motor, visceral and neurohormonal pathways. At the same time signals are transmitted to the cerebral cortex so that the emotion is perceived and recognized. If the threat to the organism continues the forces maintaining the internal equilibrium must remain active longer. This can lead to peripheral functional or even organic disturbances in the system affected. Modern anthropology which looks at man in terms of his social and interpersonal relationships emphasizes the conflicts arising out of these relationships and stresses their importance as factors in the causation of psychosomatic disorders. Thus for example retirement from an active and highly satisfying occupation can lead to a sudden deterioration in a person's health.

In order to understand the complicated multifactorial polydimensional reciprocal interplay when analysing the psychological mechanisms in a clinical picture as demonstrated by a patient it is necessary to use methods that enable an individual psychosomatic diagnosis to be established.

As von Zerssen (21) stressed in somatic medicine it has been natural that the clinical investigation of a patient is always complemented by the use of standardized physiological and biochemical examination procedures.

In psychiatry this partly became the case with the introduction of the use of psychodiagnostic tests. Very occasionally however we can see to it that the diagnostic examination of a patient having a proneness to somatic symptoms is complemented by psychological investigations which could lead to a better understanding of the genesis and the extent of the condition, the choice of therapy and often the prognosis of the particular disease.

It was the immense contribution of Professor Axel Ingelman Sundberg on whose initiative that the Psychosomatic Research Laboratory at the De-

partment of Obstetrics & Gynaecology was set up in 1973 at Sabbatsberg Hospital in Stockholm.

One of the aims and purposes from the very beginning has been to modify existing methods and elaborate some new psychodiagnostic tests which can be used by the gynaecologist and non psychologically or psychiatrically trained staff and in this way extend the diagnostic instrumentarium of the gynaecologist by one dimension.

A large proportion of patients attending particularly gynaecological clinics have neurotic or psychosomatic symptomatology. In women the reproductive system and emotions are especially closely related. It is much more important to know what sort of patient has a disease than what sort of disease a patient has. Greater attention to the needs of the person rather than of the disease would be much to advance our understanding of the disease and hopefully its relief. (1) More needs to be known about how people feel and in order to acquire this knowledge we require better techniques. An important rule in the psychosomatic diagnostic approach is the use of an individual centred technique. Any psychodiagnostic test should fulfil the following qualitative criteria:

1. it should be capable of use in the same manner for all individuals.

2. it should be capable of repetition in the same individual on several occasions in order to check the results and to follow up the development of the individual for instance during the treatment or to consider the varying influences of the individual's psychic or physical processes. (19)

3. some tests should allow a quantitative evaluation of the results and use of statistical methods.

4. the test when used in clinical practice must be easy to administer and not too time-consuming.

5. the evaluation of the test should not be too complicated. For example when used in a gynaecologic unit in order to assess psychosomatic disturbances the administration and evaluation must be practicable even for non psychologically and non psychiatrically trained staff.

6. the test must give a reliable orientation to the patient's main problems and conflicts and it should also make it possible to detect pathological changes.

In the psychosomatic Laboratory of the Gynaecologic Department Sabbatsberg Hospital we divided the psychodiagnostic methods into seven groups:

Techniques and inventories focused**case history**

are not only the life events previously experienced but also patients' attitudes and evaluations and their changes are registered

When looking through these types of scales one gets an immediate impression of the patient's basic wishes, disappointments and even some personality traits which can all give a very important background to the patient's present problems.

The following are a few examples of the evaluation scales

Mark with x your present attitude to	Satisfaction	Disappointment
sexual life	<input type="checkbox"/>	<input type="checkbox"/>
own education	<input type="checkbox"/>	<input type="checkbox"/>
own occupation	<input type="checkbox"/>	<input type="checkbox"/>
choice of partner	<input type="checkbox"/>	<input type="checkbox"/>
own children	<input type="checkbox"/>	<input type="checkbox"/>
own family life	<input type="checkbox"/>	<input type="checkbox"/>
own friends	<input type="checkbox"/>	<input type="checkbox"/>
own economic situation	<input type="checkbox"/>	<input type="checkbox"/>
realization of previous dreams and expectation	<input type="checkbox"/>	<input type="checkbox"/>

Mark with x your present life situation (mark with x) feel (mark with x)	Agree	Disagree
bitterness	<input type="checkbox"/>	<input type="checkbox"/>
joy	<input type="checkbox"/>	<input type="checkbox"/>
general satisfaction with life	<input type="checkbox"/>	<input type="checkbox"/>
disappointment	<input type="checkbox"/>	<input type="checkbox"/>
happiness	<input type="checkbox"/>	<input type="checkbox"/>
resignation	<input type="checkbox"/>	<input type="checkbox"/>
harmony	<input type="checkbox"/>	<input type="checkbox"/>
love fulfillment	<input type="checkbox"/>	<input type="checkbox"/>

Mark with x in the table below to what degree the following things are important

	Very important	Important	Less important	Unimportant
to succeed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to eat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be accepted	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be perfect	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be loved	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to have sexual desire	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be comfortable	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be secure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to love	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be happy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to make love	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to win	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to have many friends	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

to have children	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be intelligent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
to be beautiful	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

II Personality tests

The large group of personality tests and questionnaires many of which can be filled in by the patients themselves and evaluated by doctors in an often not too-complicated way offers in many cases a fairly good picture of the patient's basic personality traits. In this way one can understand much better the patient's complaints, their meaning and symbolic values and not infrequently also predict their further development.

As an example from this group we can mention the Eysenck Personality Inventory (5) which is used for measuring neuroticism and extraversion/introversion personality dimensions considered by Eysenck as basic traits. In several of our research projects (8, 9, 10) we have demonstrated the valuable use of this test both in the diagnosis and in the follow up of the treatment.

III The projective techniques

This group of tests represents a possibility for the patients to express unconscious conflicts, fears, wishes, etc. The best known and the most perfect test in this group is the Rorschach test (18).

A very simple but widely informative projective procedure has been elaborated (10). The test consists of 90 complete sentences with varying degrees of emotional relevancy for the individual patient. Example: a 24-year-old girl with secondary amenorrhea associates as follows:

<i>I feel</i>	ugly
<i>I long to</i>	look as I did 3 years ago
<i>I am most afraid of</i>	illness and death
<i>I dream about</i>	freedom
<i>A mother</i>	I feel sorry for her
<i>Being a woman</i>	frightens me
<i>I wish</i>	I were a child again
<i>My thoughts</i>	frighten me
<i>It hurts me</i>	to be the way I am
<i>I dislike</i>	femininity
<i>My mother</i>	I can never be free from
<i>Being a mother</i>	is not my dream
<i>I am not afraid of</i>	aggression
<i>A man</i>	frightens me

IV Methods for measuring depression

In accordance with Kielholtz (14, 15), Kielholtz et al. (16) and Battagry (3) we are also of the opinion that depression is a syndrome with many different etiological causes and represents a reaction of the

UTERINE ACTIVITY IN DIABETES INSIPIDUS

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Abstract In two nonpregnant women with cranial diabetes insipidus myometrial activity under different hormonal conditions was studied by intrauterine pressure record. Recordings were performed when the women were under the influence of their regular treatment with desmopressin (D-arg⁸ vasopressin (DDAVP) and when they had fully developed symptoms of their disease. Uterine activity was similar to that found in normal women under same hormonal conditions and generally did not change when symptoms of lack of vasopressin appeared. DDAVP (15-30 µg) given intranasally and lysine vasopressin (0.5 IU) given intravenously stimulated uterine activity particularly in late secretory menstrual phase. It is suggested that endogenous vasopressin is of minor importance for the induction of spontaneous uterine activity in nonpregnant women.

The two posterior pituitary hormones oxytocin and vasopressin are powerful myometrial stimulants in pregnancy particularly at term and are believed to play a physiological role in the induction of labour. It is, however, almost ineffective in the nonpregnant state. Vasopressin has little effect on the pregnant uterus at term (8) but it is a very potent stimulator of the nonpregnant myometrium (5, 7, 10). During the menstrual cycle there are also variations in myometrial sensitivity to vasopressin (2, 5, 7, 10). The effect is most pronounced at the onset of menstruation and minimal in the midcycle. These variations in sensitivity of the myometrium correspond to the variations occurring in spontaneous myometrial activity throughout the menstrual cycle (1). It is not known, however, whether vasopressin plays a physiological role in the induction of

spontaneous uterine activity in nonpregnant women. An opportunity to study this problem is offered in women with cranial diabetes insipidus in whom there is an insufficient endogenous production of vasopressin. In the present study two women with this disease were investigated as to their spontaneous uterine activity throughout the menstrual cycle.

MATERIAL AND METHODS

Subjects

Patient U. C. born 1944 had been previously healthy when her diabetes insipidus suddenly developed in 1963. There was no family history of the disease. Without medication the patient produced about 18-20 l of urine per day. Thorough investigations at the University Hospital, Lund, Sweden revealed no signs of other disorders and it was concluded that she suffered from the idiopathic type of diabetes insipidus. She was given replacement therapy with desaminocystine¹ D-arg⁸ vasopressin (DDAVP) (Minirin[®] Ferring, Sweden) 0.05 µg taken intranasally 3 times per day. As a result of this treatment her daily urine production was reduced to normal (volumes 0.9-1.7 l/day, urine osmolality 450-780 mOsm/kg H₂O) and she could lead a normal life. The patient had been pregnant twice since the start of the disease in 1968 and 1971 and she delivered spontaneously 3 and 5 days after expected time respectively without any complications. In the puerperium she experienced slightly painful uterine contractions after the second delivery but not after the first. She nursed her second child for a period of 3 months after the delivery. Both her infants are at present healthy and developing normally.

During the present investigations she had regular menstrual cycles of 28-30 days with menstruation of 4 to 5 days duration. Her basal body temperature chart showed a biphasic pattern. Progesterone and oestradiol estimations on plasma samples taken the day of the recordings were within normal limits (13) indicating that she had ovulatory menstrual cycles. Gynecological examination

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cle (Fig 1) and more pronounced at the end of the secretory phase (Fig 2) or during combined oestrogen and gestagen therapy (Fig 3)

DDAVP had a slight stimulatory effect on uterine activity appearing within 5 min after intranasal administration (Figs 1 and 2). The stimulatory effect of LVP given intravenously was more pronounced especially in terms of frequency of contractions and of uterine tone (Figs 2 and 3). The local endometrial blood flow decreased after administration of vasopressin (Fig 3) in agreement with what has been found in normal women (2).

After administration of DDAVP or LVP the specific gravity and volume of urine produced were both within normal limits.

DISCUSSION

The possible physiological role of vasopressin in the induction of uterine activity in nonpregnant women has previously been studied by infusion of iso-oncotic solutions in order to inhibit the endogenous secretion of vasopressin (9). In those experiments a complete inhibition of myometrial activity during menstruation was obtained in the majority of the investigated women. It was suggested therefore that vasopressin may be a physiological stimulus for uterine contractions during menstruation. A more direct way of analyzing the effects of a lack of vasopressin is to study uterine activity in women with diabetes insipidus. This also excludes other possible effects due to the infusion of large amounts of fluid.

The women in the present study had previously been shown to have an insufficient vasopressin production as judged from the results of water deprivation tests and inability to increase their urine osmolality in response to hypertonic saline infusion (Carter Robbins test). Furthermore administration of vasopressin could completely relieve their symptoms.

The woman with idiopathic diabetes insipidus receiving regular treatment with DDAVP seemed to have a quite normal reproductive function. She had been delivered of two children spontaneously since the start of the disease and had nursed her second child. She had also spontaneous ovulatory menstrual cycles. The other woman had an insufficient endogenous production of ovarian hormones and in addition her substitution therapy seemed to be insufficient as menstruation did not start until 5

days after stopping the cyclical treatment. Furthermore her uterine activity the day before the onset of the withdrawal bleeding was minimal.

The uterine activity in the two women during the time they still were receiving regular treatment with DDAVP did not seem to differ from that seen in women in the same ovarian hormonal state. The symptoms of diabetes insipidus had developed indicating a lack of vasopressin, this generally does not influence the recorded spontaneous uterine activity. The only exception to this was one recording in the patient with idiopathic diabetes insipidus investigated during early secretory menstruation when the uterine activity decreased.

In conclusion the results of the present study suggest that endogenous vasopressin is of great importance for the induction of spontaneous uterine activity in nonpregnant women. Other uterine stimulants such as E and F prostaglandins are probably factors of major importance at least around the onset of menstruation.

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FUNCTIONAL ROLE OF AN ADRENERGIC SPHINCTER IN THE FEMALE URETHRA OF THE GUINEA PIG

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Abstract By means of a microtransducer catheter the urethral pressure profile was recorded in female guinea pigs. The maximum urethral pressure averaged 24 mmHg (range 18-27 mmHg) and was found 4 to 7 mm from the urethral junction. Fluorescence microscopy of the urethra showed an irregular distribution of fluorescent nerve terminals in the most proximal part of the organ. In distal direction there was a continuous increase in the number of adrenergic nerves with a maximum 4 to 6 mm from the internal meatus corresponding to the maximum intraurethral pressure point. In this region a dense plexus of nerves supplied thick muscle coats of smooth muscle and an outer circular and an inner longitudinal orientation. The findings suggest that a specific region of the female guinea pig urethra may serve as an internal sphincter.

Evidence has been presented suggesting the existence of a functional sphincter in the proximal part of the urethra both in different animal species and in man (8, 11, 13, 15, 18). A great number of pressure profile measurements in human female urethra have clearly demonstrated an intra luminal pressure peak at a distance of 11-12 mm from the inner meatus of the urethra (1). This finding confirms the existence of a functional urethral sphincter. No morphological arrangements convincingly showing a sphincter mechanism have however so far been found (1, 5, 17).

Several studies have stressed the importance of the adrenergic nervous system for the function of the internal sphincter. Histological investigations of the adrenergic innervation of the urethra of cat, dog and rat have shown a rich occurrence of adrenergic nerve terminals (2, 14, 16) especially

abundant in the muscle of the proximal urethra (14). The role of the adrenergic innervation for the sphincter function has however not been settled.

The aim of the present investigation was to find out whether a relationship exists between the adrenergic innervation pattern and the intraurethral pressure profile.

MATERIAL AND METHODS

The study was performed on 11 female guinea pigs weighing 300-480 g.

Pressure recordings

Technique The intravesical and intraurethral pressures were recorded by means of a previously described technique for simultaneous methocystometry and urethral pressure profile measurement (1). The intraluminal pressures are recorded by means of a micro-transducer enclosed in a semisflexible dacron catheter. The active pressure sensor area of the micro-transducer is only 0.75 mm² and the outer diameter of the catheter is 1.6 mm. The frequency response of the recording system is more than 1000 Hz (19). After amplification the pressure signals were recorded by an ink jet recorder (Siemens-Elema No 81).

Experimental procedure After anesthesia with pentobarbital the recording catheter was introduced transurethraly so far that the micro-transducer at the tip of the catheter was placed in the bladder. The intravesical pressure was recorded for about one minute. The catheter was then connected to a specially designed withdrawal instrument (1). Using this instrument the catheter including the micro-transducer was pulled backwards at a constant rate of 3.6 mm/sec. By this manoeuvre the intraluminal pressure was continuously recorded throughout the entire length of the urethra from the inner to the outer meatus—the urethral pressure profile (Fig. 1).

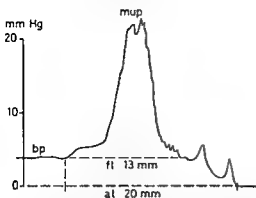


Fig 1 Representative urethral pressure profile from one of the guinea pigs bp Bladder pressure mup maximal urethral pressure fl functional length of the urethra al absolute length of the urethra

Fluorescence histochemistry

After completing the pressure recordings in the intact animal the abdomen was opened by a lower midline incision and the symphysis was transected to expose the urethra. The recording device was again inserted and could easily be recognized through the thin translucent wall. By moving it through the lumen it was possible to confirm the exact anatomical point corresponding to the pressure maximum. A 5 mm long portion of the urethra including this point and two additional adjacent pieces of the same length distally and proximally were dissected out and immediately frozen in a propane propylene mixture at the temperature of liquid nitrogen.

In addition to the pieces obtained from these 7 animals the entire urethra was removed from a further 4 untreated animals and frozen in 3 pieces of equal length.

After freeze drying the preparations were exposed to gaseous formaldehyde at $+80^{\circ}\text{C}$ for 1 hour to visualize the adrenergic transmitter, noradrenaline (9). Following paraffin embedding in *vacuo* 6 μm thick serial transverse sections were prepared and mounted in Entellan (Merck).

fluorescence microscopy. For further technical details Bjorklund et al. (3).

RESULTS

Fluorescence histochemistry

The smooth musculature in the entire length of the urethra was supplied by adrenergic nerve terminals characterized by a beaded (varicosed) appearance and emitting an intense green fluorescence under the optical conditions used. Serial sectioning of the organ revealed a clear regional variation in the amount of muscular nerve fibres: the most proximal part was characterized by an irregular distribution of scattered fluorescent terminals together with axons enclosing several small blood vessels (Fig. 2). In a distal direction there was a continuous increase

in the number of adrenergic nerves until was reached approximately 4–6 mm. In this region (Fig. 2) a dense plexus supplied thick smooth muscle coats as an outer circular and inner longitudinal layer. This was followed by a fairly rapid decrease in nerve density in the distal part (Fig. 2). Here again the fluorescent terminals were arranged in a more irregular fashion reflecting the anatomical arrangement of the smooth muscle fibres. The distal part of the urethra contained no shaped enterochromaffin-like cells in the epithelium. Occasionally it was seen that the adrenergic axon terminals were located underneath this epithelium.

Pressure recordings

In all animals the intravesical pressure was low and stable and varied between 2 and 5 mmHg (mean 4 mmHg). The intraurethral pressure varied in different parts of the organ. The highest intraluminal pressure was recorded in midurethra 4 to 6 mm (mean 5 mm) from the inner meatus where the maximal pressure amplitude was found to average 27 mmHg (range 18 to 27 mmHg). The functional length of the urethra, i.e. the region where the intraurethral pressure exceeds the intravesical pressure, varied between 12 mm and 16 mm (mean 14 mm). The absolute length of the urethra, i.e. the region where the intraurethral pressure exceeds atmospheric pressure, was difficult to evaluate in some cases but varied between 16 mm and 23 mm (mean 18 mm).

Fluorescence microscopy of that part of the urethra in which a pressure maximum was recorded showed an exact correspondence with the most pronounced supply of adrenergic nerves in the smooth muscular wall (Fig. 2).

DISCUSSION

The sphincter function of the lower urinary tract is still a matter of discussion. Thus the existence of an internal sphincter is not established and it is not settled whether the bladder neck or any particular part of the urethra can serve as the internal sphincter. Lapides (12) concluded on the basis of experiments performed mainly on dogs that the vesical sphincter is a tubular muscular structure synonymous with the posterior urethra in the male or the entire urethra in the female. Gleason et al.

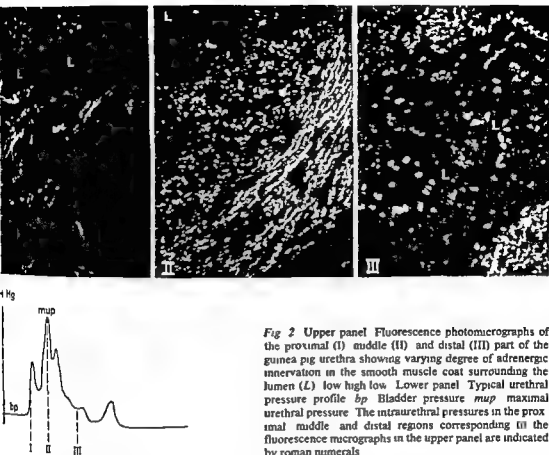


Fig 2 Upper panel Fluorescence photomicrographs of the proximal (I) middle (II) and distal (III) part of the guinea pig urethra showing varying degree of adrenergic innervation in the smooth muscle coat surrounding the lumen (L) low high low Lower panel Typical urethral pressure profile *bp* Bladder pressure *mup* maximal urethral pressure The intraurethral pressures in the proximal middle and distal regions corresponding to the fluorescence micrographs in the upper panel are indicated by roman numerals

studying the urethral pressure profile in man suggested that the extreme sensitivity of continence to urethral pressure must localize the continence mechanism to the urethra. They found a specific zone of the urethra to be critical in maintenance of continence and that the bladder neck did not appear to be a critical part of the continence mechanism.

Studies on the distribution of adrenoceptors and adrenoceptors in the isolated human urethra and the effects of drugs stimulating these receptors suggested that the response to adrenoceptor stimulating agents was similar throughout the organ including the urethrovesical junction (6). Thus there was a predominance of contraction mediating α -adrenoceptors relaxation mediating β adrenoceptors could be demonstrated. The response to adrenoceptor stimulation on the other hand seemed to be insignificant. In all parts of the urethra there was a scanty but rather uniform supply of adrenergic nerves (7). These findings sup-

The information is scarce concerning the

ences in the anatomical structure of the urethra that may exist between different experimental animals. Little is also known about the distribution of adrenoceptors and cholinceptors and the sensitivity to drugs stimulating these receptors. This should be borne in mind when studies on the urethral function in animals are compared and especially when comparing data from animal studies with those obtained in man.

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PUBOCOCCYGEAL REPAIR AD MODUM INGELMAN SUNDBERG

*A Retrospective Investigation with 10-20 Years Time
of Observation*

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over Fifty-one women operated on for stress incontinence with pubococcygeal repair ad modum Ingelman Sundberg between 1955 and 1965 were investigated urologically. The time of observation was accordingly at 10 years in every case. An evaluation was made of operation method, the distribution of recurrences during the postoperative period and possible side effects. 11 women had symptoms of at least degree I stress incontinence and thirteen showed signs of incontinence at examination. Only four had both symptoms and signs of incontinence. The cure rate from a symptomatic point of view was 84% and from the technical aspect 75%. Most severe recurrences occurred in the first postoperative year. Thereafter the recurrences were evenly distributed. The only side effect in the long run was a tendency to

abdominal increase of pressure. Several hypotheses concerning the genesis of stress incontinence have been presented and given rise to a growing variety of operation methods.

At the beginning of the 1960s Enhörning (7) using simultaneous urethrocystometry showed that a sudden rise of the intra abdominal pressure normally is transmitted not only to the bladder but also to the proximal third of the urethra which is to be considered an intra abdominal organ. In cases of stress incontinence the bladder neck and the proximal urethra are lowered dorso-caudally so that a sudden pressure rise e.g. at coughing only reaches the bladder and not the urethra. The urethra cannot produce a sufficient pressure when in a lowered position in spite of the fact that the intraurethral pressure at rest is always higher than the corresponding bladder pressure. Thus according to Enhörning stress incontinence appears for hydrodynamic reasons. It is still unclear whether a lowered bladder neck alone explains the leakage. Insufficiency of an internal and/or external sphincter (18, 19, 22), funneling of the proximal urethra (1, 12, 21, 27, 28), an abnormally short urethra (20) and a more or less pathological posterior urethrovesical angle (9, 10, 16, 17, 26) have also been put forward as plausible explanations. As basic causes of these changes of the uro-genital anatomy a general insufficiency of the connective tissue, menopause, pregnancy and vaginal deliveries have been reported (3, 6, 8). However, no direct correlation exists between parity and the occurrence or severity of stress incontinence and the dysfunction occurs even in nulliparous women.

Stress incontinence is a dysfunction rather than an injury and occurs only in homo. It involves involuntary urinary leakage at a sudden rise of the intra-abdominal pressure. It can be classified as follows (15):

- type 1 Urinary leakage only at coughing or sneezing
- type 2 Leakage also at changing position, moving fast or walking up stairs
- type 3 Leakage even at minimal straining in the standing position

During the last decades the genesis of stress incontinence has been thoroughly investigated and studied. Interest has focused on the anatomical and physiological changes in the bladder neck and the proximal urethra during voiding and on pressure variations in the bladder and the urethra at a sudden

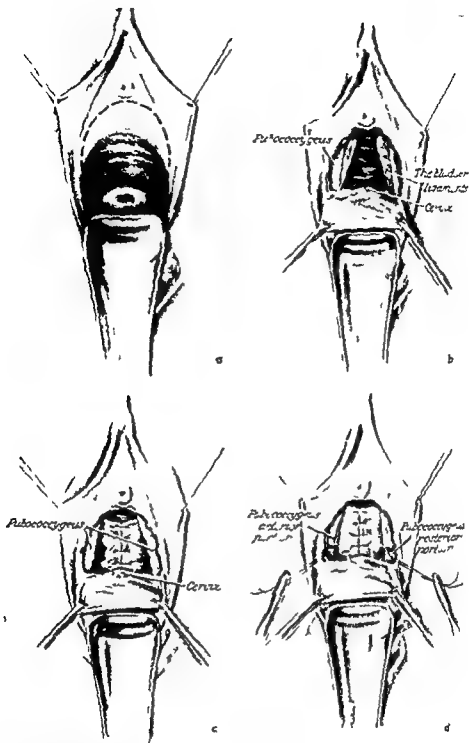
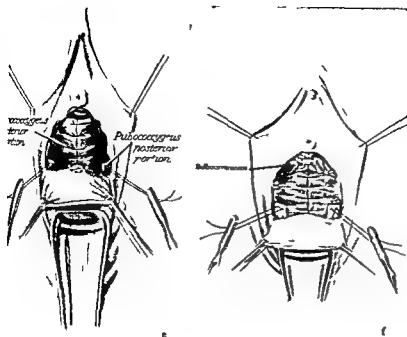


Fig 1a-d For explanation see text. By permission of Axel Ingelman Sundberg.

Since a lowered position of the bladder neck seems to be the main reason for stress incontinence it is essential to choose an operation method that elevates this region. In urethropexia ad modum

Marshall Marchetti Krantz Kelly's operation and various types of sling operations. The pubococcygeus repair operation ad modum Ingelman Sundberg is from a theoretical and physiological point of



1-e-f For explanation see text By permission of Axel Ingelman Sundberg

is suitable for severe degrees of stress incontinence. It makes use of the patient's own muscles (the pubococcygeus; the medial portions of the levator ani muscles) which are sutured under the bladder neck as a hammock with contractile ability. At voiding urine this hammock is supposed to contract and elevate the bladder neck and the proximal urethra in an antero-cranial direction.

Previous retrospective investigations performed on stress incontinent patients after other operations have mostly been made at an observation time of 5 years or less. Since recurrences are sometimes seen later it is of interest to study a group of patients who have all been observed for 10 years or more after the surgical treatment. The patients now investigated had all been operated on according to the standard method for pubococcygeal repair adopted by Ingelman Sundberg between 1955-1965. The observation time is 10-20 years. The aim of the investigation was to evaluate the effect of this operation method and the distribution of the recurrences during the postoperative period. The main indication for operation was stress incontinence but cases of incontinence caused by scar tissue were also represented. Some of these patients had

already been operated on several times because of stress incontinence. The results are not to be compared with those of other investigations where cases of pure stress incontinence have been selected and operated on for the first time. In 1952 Ingelman Sundberg (14) made a follow up of 82 patients operated on with pubococcygeal repair on the indication stress incontinence. The observation time was 2-5 years. Seventy seven patients were cured and 5 improved. This good result was achieved even though 4 patients had a vaginal delivery during the observation period. The cure rate in this follow up was 94%. For some patients the observation time was short but still the method seemed to be suitable for treatment of stress incontinence. In 1964 Christensen & Østergaard (5) made a follow up on 40 stress incontinent patients operated on with pubococcygeal repair and with an observation time of 0.5-9.5 years and got equally good results. The same year Ochshorn (24) reported similar results.

MATERIAL AND METHOD

Included were 51 patients who underwent the pubococcygeal repair operation between 1955-1965. Excluded

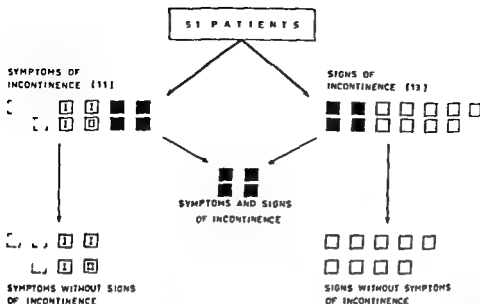


Fig 2 Recurrences 10-20 years after pubococcygeal repair. The Roman numerals inside the squares signify the

degree of incontinence. The dotted squares mean 1st degree of incontinence periodically only.

were 88 patients mostly living in distant parts of the country who could not be traced or had no opportunity to come for an examination. All patients had been thoroughly examined uro-gynecologically before the operation. This meant neurological control of the S1-S4 dermatomes, sphincterometry (ad modum Leander), urethrocytostomy, cystometry (Lewis) and Bonney's test.

The mean age at the operation was 66 years ($r=86-4^{\circ}$) and the mean parity 2 ($r=4-0$). The mean duration of stress incontinence of degree 1 before operation was 9 years, degree 2 2.5 years, degree 3 0.5 years.

Five patients had been operated on previously because of incontinence. Another 5 patients had been operated on with the Manchester operation.

A small group of 14 patients operated on during the same time with plasty of the bladder floor in a suture only of the bladder ligaments (pelvic fascia) was also examined to investigate if there were any differences regarding the frequency of recurrences. The mean age and parity for this group were approximately the same as in the group operated on with the pubococcygeal repair.

Operative technique ad modum Ingelman Sundberg (Fig 1a-f)

An arcuate incision is made below the external urethral meatus (Fig 1a). The anterior vaginal wall is dissected from the urethra and the bladder floor up to the cervix (Fig 1b). The bladder ligaments are dissected and sutured in the midline from the proximal urethra to the cervix to which the innermost suture is fixed (Fig 1c). Continence is checked with 300 ml bladder volume at repeated coughs. The pubococcygeal muscles are dissected and totally divided just below the middle (Fig 1d). The anterior portions of the pubococcygeal muscles are sutured under the bladder neck and the proximal part of the

urethra so that a muscle sling is formed (Fig 1e). The bulbocavernosus muscles are sutured in the midline without division as a support for the distal part of the urethra (Fig 1f). The posterior portions of the pubococcygeal muscles are sutured bilaterally to the ischio cavernosus muscles. The anterior vaginal wall is sutured at the isthmus; a suprapubic catheter is applied as well as a vaginal tamponade.

Experimental performance

The patients were examined in the lithotomy position and a gynecological status. The degrees of cystocele, urethrocele and rectocele were noted as well as the degree of downward rotation of the anterior vaginal wall on coughing. The presence of urinary leakage at a bladder volume of 300 ml at repeated coughs was checked. In cases of leakage Bonney's test was performed. Preferably leakage should have been controlled also in a standing position but this was impossible in some cases because of the fragility and high age of the patients. Residual urine volume was estimated. The history was thoroughly penetrated concerning the time for and degree of recurrence. Besides the case reports of the patients were checked regarding previous operations, degree and duration of the incontinence before the pubococcygeal repair.

RESULTS AND DISCUSSION

Eleven out of 51 patients reported symptoms of incontinence during the postoperative period (Fig. 2). Only in 4 cases could urinary leakage be confirmed at examination. One of the remaining seven patients had got symptoms similar to those before the operation and the others had symptoms of a

Table 1 *Time of recurrence*

Years after op	No of recurrences
0-3	6
4-5	1
6-10	2
10-20	2

signs are recurrences although most of them did not complain of incontinence symptoms. On this premise the cure rate was 75%.

In the group of patients with signs of urinary incontinence 7 out of 13 had an abnormal lowering of the vaginal wall at straining. The corresponding number for those with only symptoms was 1 out of 7 and for the cured patients 2 out of 31. Thus a clear correlation existed between signs of urinary leakage and lowering of the vaginal wall. As the lowered position of the bladder neck and the proximal part of the urethra has long been considered to be the main cause of stress incontinence (2, 4, 7, 11, 23, 25) it seems doubtful if the patients with only symptoms and without any lowering of the vaginal wall should be regarded as recurrences. Also considering the results of the psychodiagnosis in investigation these patients are possibly not to be affirmed stress incontinence recurrences. If the patients with both an abnormal lowering of the vaginal wall and signs or severe symptoms of incontinence are counted the cure rate was 85%.

The frequency of rectocele after the pubococcygeal repair was high. The reason for this could be that the medial portion of the m. levator ani had been cut. A contributing factor could be that the anterior vaginal wall is very firm postoperatively and that because of this the posterior wall gives away at straining. In 22 of the 51 patients the pubococcygeal repair was combined with a posterior colporaphy because of rectocele. Eleven of these 22 patients got recurrences of their rectocele. Another 17 in whom no posterior colporaphy had been done got rectocele during the observation time. As stress incontinence is probably at least partly a kind of insufficiency of the connective tissue one could presume the frequency of rectocele to be higher in these women than normally. Because the incidence of rectocele was almost the same independently of the patients being operated on for rectocele or not it seems logical to combine

pubococcygeal repair with posterior colporaphy only in cases of troublesome rectocele.

No certain conclusions could be drawn concerning the possible correlation between parity and stress incontinence. The mean parity and the distribution of nulliparous women were the same in the group with symptoms of incontinence and the group with signs.

The small group of patients operated on with only bladder ligament suture had a symptomatic cure rate of 43% and a technical cure rate of 64%. When the cure rate was based on an abnormal lowering of the vaginal wall combined with signs or symptoms of incontinence it was 77%. The recurrences were evenly distributed ranging from 1 to 18 years with a mean of 7 years. It is therefore possible that the muscle sling is necessary to obtain a good and lasting result.

CONCLUSION

This investigation aimed at studying what happens in the long run (10-20 years) after pubococcygeal repair ad modum Ingelman Sundberg. There are two ways of estimating the cure rate: from the patient's point of view or from a technical aspect. The cure rates were 84% and 75% respectively. Most of the recurrences occurred during the first postoperative year. These included the most serious cases which since then several reoperations have been performed without success. The other recurrences were evenly distributed during the long observation time. The only side effect of the operation in the long run seemed to be a tendency to rectocele. It is useless to combine the pubococcygeal repair with a posterior colporaphy unless trouble from the rectum is present. Comparison with a small group of patients operated on with only plasty of the bladder ligaments showed that in the long run the muscle sling was an advantage for the cure rate. Considering these results and the long observation time the pubococcygeal repair method seemed to be most suitable for patients with serious urinary stress incontinence and affords a good chance of curing the patients for life.

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INGELMAN SUNDBERG OPERATION FOR URINARY INCONTINENCE

Our Experience

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The authors analyse the indications for the Ingelman Sundberg operation considering pelvic anatomy, previous surgery and the age of the patient. The operation is described with pre- and postoperative management. Associated operations such as penneorrhaphy, the amputation of the uterine cervix are mentioned. By a study is made of the experience of the Gynecologic Clinic of the Catholic Faculty of Medicine concerning the postoperative problems that have arisen. The results obtained in the cure of urinary incontinence. The surgical treatment of urinary incontinence to lesions of the pelvic floor presents several difficulties with a high incidence of recurrence of symptoms.

In search of a solution for the problem many surgical techniques have been proposed, some involving the supra pubic approach such as the Marshall-McChesney-Kranz and Burch techniques, others the vaginal route in which the Kelly operation described in 1911 is the original technique and the results of others with lifting of the base of the bladder and lengthening of the urethra.

The Ingelman Sundberg operation involves the division of the pubo-coccygeus along with the principles described by Kelly. The original incision curves transversely across the vagina with the concavity directed posteriorly and inferiorly and the pubo-coccygeus muscles are completely divided in order to construct a muscular belt under the base of the bladder.

The use of the pubo-coccygeus had previously been strongly recommended for reducing the number of recurrences. Suture of the pubo-coccygeus muscles together in the mid line was described by Martinus by Kennedy and by other authors. However there is a tendency to separation when the muscles contract. Previously partial division of the pubo-coccygeus had been proposed by

several surgeons such as Squier, Franz and Tausig. The problem is that in time the muscle fibres lose their contractility and become atrophied.

Ingelman Sundberg, taking into account the fact that the pubo-coccygeus receives blood vessels and nerves from both sides, perfected the complete division of the muscle and the shaping of a belt passing under the base of the bladder by suturing the two parts together. This belt is under voluntary control and therefore aids the control of micturition. At the Gynecologic Clinic of the Catholic Faculty of Medicine of Porto Alegre (Chairman Prof J Gomes da Silva) the Ingelman Sundberg operation was introduced for the surgical treatment of urinary incontinence when associated with urethrocele or small cystocele and when the patient has already been subjected to a hysterectomy.

We consider as contra indications for this technique the presence of large cystoceles where the incision is unsuitable for colporrhaphy, uterine retroversion and a history of previous vaginal surgery for uterine prolapse.

MATERIAL AND RESULTS (25 operations)

Indications

Small cystocele with urethrocele 22 cases (88%)
Large cystocele with urethrocele 3 cases (12%)
Incontinence of urine 25 cases (100%)
Previous hysterectomy 2 cases (8%)

Age

<20 years 1 case
20-30 years 4 cases
30-40 years 12 cases
40-50 years 7 cases
50-60 years 1 case

Associated operations

Amputation of the uterine cervix 5 cases (20%)
 Pelveorraphy 21 cases (88%)

Complications

Urinary infection 5 cases (20%)
 Urinary retention 3 cases (8%)
 Hemorrhage 1 case (4%)

Our results during a maximum period of three years

Cure 23 cases (92%)
 Recurrence 2 cases (8%)

CONCLUSION

In our experience from the technical point of view the surgery presents the following advantages: (1) Utilization of the voluntary mechanism for urinary control. (2) The incision favors approach to the

urethra avoiding large vaginal incisions in cases involving urethrocele without cystocele. (3) It does not use the cervix as a point of reference and traction, which makes it suitable in cases of patients who have undergone hysterectomy.

The post-operative period requires special care in the drainage of the bladder which is achieved by a supra pubic drain which remains open for the first 24 hours. After this period the drain is closed and spontaneous micturition encouraged. Our average period during which the drain is kept in place has been three days.

The hospitalization period has varied from five to nine days. We have emphasized post-operative exercises to contract and relax the pubo-coxigenal muscles.

Submitted for Festschrift

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INTRA-VESESICAL NERVE RESECTION FOR DETRUSOR DYSSYNERGIA

The Ingelman Sundberg Operation

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In 1949 a paper was published in Acta Obstet Gynecol Scand (38 487 1959) by Axel Ingelman Sundberg (1) entitled "Partial Denervation of the Bladder—a new operation for the treatment of Urge Incontinence and similar conditions in women". The basis for the operation was laid in the initial sentence "The function of an autonomous neurogenic bladder is usually superior to that of a neurogenic bladder where higher centers are still partially functional". His operation consisted of transection of the ganglionic pelvic nerves near the inferior surface of the bladder and involved division of both the sympathetic and parasympathetic nerves.

In a preliminary test to the operation Ingelman Sundberg employed continuously recorded retrograde water cystometry and recorded the first desire to void bladder capacity sphincter pressure and residual urine volume. Following the initial cystometric study he injected 0.25% xylocaine with epinephrine 0.005% into one super lateral vaginal fornix 1 cm lateral to the cervix at a depth of 3 cm. After waiting 5 minutes the cystometric study was repeated. If all inhibited bladder contractions stopped by unilateral injection the investigation was stopped. However if uninhibited contraction continued or if bladder capacity was still too small xylocaine injection was made into the opposite fornix. If a favorable subjective response was obtained and if residual urine volume did not exceed 150 cc the patient was considered to be favorable for unilateral intra-vesical partial nerve resection. He employed the operation 32 times for various types of urinary incontinence including urge incontinence, infectious incontinence including interstitial cystitis, uninhibited neurogenic bladders, encyresis, cerebral lesions including multiple sclerosis and myelomeningocele. In retrospect Ingelman Sund-

berg considered the main indication for the operation was for patients with uninhibited neurogenic bladders and suggested that the other conditions could probably be best managed by specific anticholinergic drug therapy.

In preparing an article for this Ingelman Sundberg's Festschrift in honor of his many years of service as Editor for Acta Obstet Gynecol Scand I have chosen to report my results on the operation of partial resection of the intra-vesical nerves for urinary incontinence caused by detrusor dyssynergia (2) as portrayed by direct electronic urethrocystometry.

Direct electronic urethrocystometry was first reported in 1963 by Hodgkinson & Cobert (2). The technique was devised to continuously monitor bladder and urethral pressures as the bladder naturally filled with urine from the time the bladder was empty until it became subjectively full.

To test for bladder irritability two provocative tests for detrusor dyssynergia are performed. The first provocative test is done immediately after the catheters are installed when the bladder is essentially empty. To perform this test the patient is asked to stand erect. She is then asked to forcibly cough 3 times at 30 second intervals. Following coughing she is then asked to stand on her toes and forcefully jounce downward on her heels on 3 separate occasions at 30 second intervals. She is then placed flat on the table allowing the bladder to fill naturally and she is asked to report when she experiences a natural sense of urgency to void. At this time the second provocative test similar to the first is performed. Following this she enters the bathroom and behind the closed door is asked to

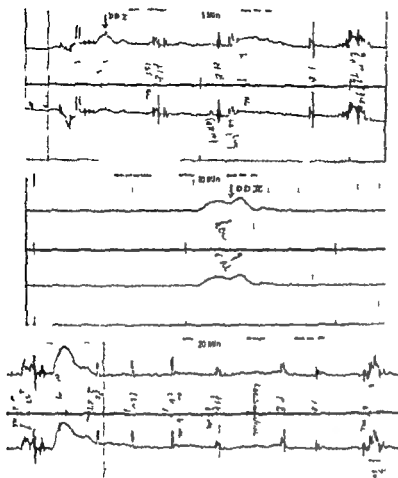


Fig 1 In all frames the upper tracing represents intravesical pressure and the lower tracing mid urethral pressure

Detrusor Dyssynergia Type I and II Type I Detrusor Dyssynergia is an uncontrollable detrusor contraction provoked by some skeletal muscle activity such as coughing. Type II is an uncontrollable detrusor contraction occurring spontaneously without skeletal muscle activity. It is apparently provoked when the bladder urine reaches a certain critical volume peculiar to each patient (Indicated by arrows)

void. During voiding the pressures in the bladder and urethra are recorded. After voiding the volume of residual urine and the total bladder capacity are measured.

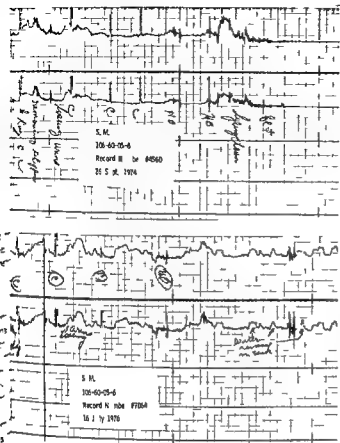
Using this simple technique several basic observations fundamental to normal urinary control were observed. *First* it was observed that in normal continent women there is no increase in either bladder or mid urethral pressure from the time the bladder is empty until it is subjectively full. *Second* no detrusor contractions can be provoked by coughing or jouncing on the heels indicating complete suppression of detrusor auto contractility. *Third* increases in intra abdominal pressure induced by coughing are directly transmitted to the bladder and mid urethra. *Fourth* the sensation to void is incidental to bladder urinary volume and not to increased intra vesical pressure.

The above conditions always prevail in normal adult females as well as in patients with anatomic stress urinary incontinence. However in the

screening of women complaining of urinary incontinence a certain number were discovered to be losing urine incidental to abnormal detrusor contractions. Because the uninhibited detrusor contractions resembled those observed in patients with neurologic bladders intensive neurologic investigations were performed. In a small percentage of cases a definite neurologic deficit was detected. These patients were assigned to a category of neurologic disease and were excluded from the study. The vast majority of patients observed to lose urine because of uninhibited bladder contractions were proven to be free of both neurologic and urologic disease. Because of this they were assigned to a diagnosis of detrusor dyssynergia of unknown etiology.

IDIOPATHIC DETRUSOR DYSSYNERGIA

Continued study of this group of patients has suggested the following observations



Figs 2-3 Patient S.M. was first observed in 1974 with mild and usually controllable detrusor dyssynergia Types I and II. She gained some relief with anticholinergic drug therapy. However, her disability gradually increased to the point where she was constantly soaking wet. Repeat direct electronic urethrocystometric study performed in 1976 (Fig 3) showed marked increase in the degree of detrusor dyssynergia.

Detrusor dyssynergia as disclosed by direct electronic urethrocystometry is manifest as two types. Type I is an uncontrollable detrusor contraction occurring as the result of some skeletal muscle activity such as coughing or jouncing on the heels. There is evidence in provocative tests 1 and 2 (Fig 1). Type II detrusor dyssynergia occurs as a spontaneous unprovoked detrusor contraction when the patient is lying flat on the table. It appears as a crescendo-diminuendo type of smooth muscle contraction producing a pressure rise in both the bladder and mid urethral leads. The increased pressure reaction causes a spontaneous flow of a small amount of urine (50-100 cc). The detrusor contraction is apparently triggered by a critical bladder volume. Once the contraction ceases, bladder stability again subsides until the critical volume is again reached when the detrusor contraction again occurs. This sequence is repeated at regular intervals as long as the bladder and urethral pressures are recorded (Fig 1).

Detrusor Dyssynergia both types I and II are

purely motor phenomena because a sense of urgency to void never accompanies the urine loss.

It appears likely that detrusor dyssynergia is a condition peculiar to women. If males lose urine on the basis of abnormal detrusor contractions, some neurologic abnormality can usually be assigned.

In patients with detrusor dyssynergia certain pathologic urologic manifestations may be present. Persistent residual volumes of 75 to 150 cc are usually present on cystoscopic examination. Fine trabeculations of the bladder are usually evident. This latter observation has at times led to the diagnosis of vesico-urethral obstruction. On this basis sometimes vesical neck resection has been performed. At times this has led to complete urinary incontinence.

Patients with detrusor dyssynergia usually increase in severity with the passage of time (Figs 2 and 3).

About 50% of patients with detrusor dyssynergia are responsive to anticholinergic drugs such as Propantheline (Probanthine, Searle) and

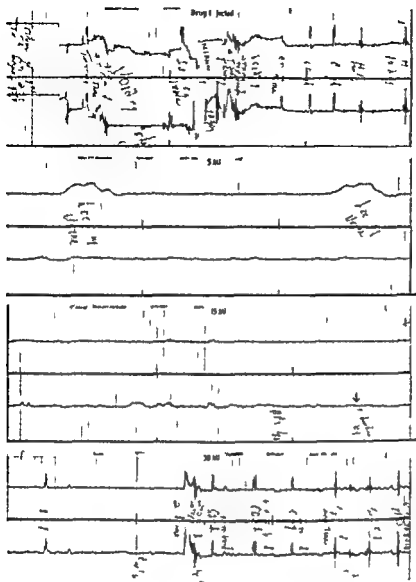


Fig 4 Detrusor dyssynergia in patients I and II completely responsive to Probanthine 15 mg IV. The time injection is indicated by the arrow. Note complete freedom of detrusor contractions during provocative test no. 2 in the bottom frame. (The urethral lead failed to function adequately during this test.)

Company Chicago Ill.) Such patients can frequently be managed by oral medication with establishment of excellent urinary control (Fig 4).

7 About 50% of patients with detrusor dyssynergia respond poorly or not at all to anticholinergic drugs. These are the patients for whom the operation of Ingelman Sundberg resection of the infravesical nerves may be considered.

DIRECT ELECTRONIC URETHRO VESICAL CYSTOMETRY AND DETRUSOR DYSSYNERGIA

Although Ingelman Sundberg advantageously employed retrograde standard water cystometry

continuously recorded for the study of his new direct electronic urethrocystometry has been shown to have many advantages. Although standard water cystometry and the newly introduced explosive gas cystometry will provide an unstable bladder muscle to vigorously contract, neither of the methods are sufficiently discriminative to differentiate bladder instability, incidental cystitis, nerve dysfunction, muscle dysfunction, and psychogenic bladder dysfunction.

In patients discovered to be losing urine because of detrusor dyssynergia the following investigation procedure is followed at Henry Ford Hospital:

1 The patient is referred to the neurologist for a comprehensive neurologic examination if a

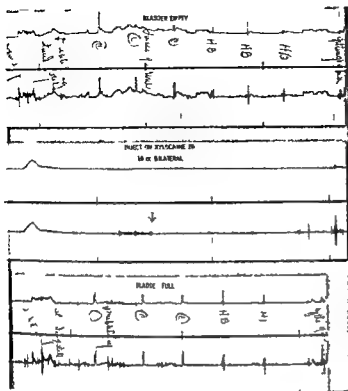


Fig 5 Detrusor dyssynergia Types I and II. Injection of 1% Diethylaminoacet 2-6-xylidide with 0.0005% epinephrine 10 cc bilaterally 1 cm lateral to the cervix 3 cm depth (indicated by arrow). Note complete suppression of detrusor contractions subsequent to time of injection (Preoperative to partial infravesical nerve resection. Same patient as in Fig 6).

Urologic deficit is discovered. Investigative procedures are continued.

The patient is referred for comprehensive urologic investigation. If no specific urologic lesion is discovered, investigation is continued.

With the above two studies declared negative, repeat direct electronic urethrocytometric study is performed. The test is allowed to continue until simultaneous detrusor pressure contractions are observed. At this time the patient is tested to determine her reaction to an anti-cholinergic drug. Propantheline 15-30 mg is injected intravenously. If the patient responds, the investigation is stopped. She is given a trial with oral anti-cholinergic drugs: Propantheline Bromide (Probanthine, Scarle Company, Chicago, Ill.), Imipramine Hydrochloride (Imipramine, Wyeth Company, Philadelphia, Pa.), and Sodium Diphenylhydantoin (Dilantin, Davis Company, Detroit). If a favorable clinical response is obtained, an appropriate dosage schedule is worked out and no additional treatment is necessary (Fig 4).

If the patient fails to respond to anticholinergic drugs, the direct electronic urethrocy-

tometric test is again repeated. Once the detrusor contractions have been observed to occur, an injection is made of 10 cc of 1% Diethylaminoacet 2-6-xylidide with 0.0005% epinephrine 1 cm supero-lateral to the cervix at a depth of 2 to 3 cm. If the patient is responsive to local anesthesia, the abnormal detrusor contractions will be immediately suppressed (Fig 5). Patients who respond in this fashion are considered to be candidates for partial infravesical nerve resection.

Although patients who have been properly investigated will usually obtain an excellent response to infravesical nerve resection, it must be remembered that the operation is purely empirical. In the occasional patient the operation will fail completely while in others it will be completely successful. Occasionally the relief obtained by the operation will be variable and at other times an initially successful operation will be followed by gradual recurrence. For these reasons, a complete explanation of the possible results must be given to the patient beforehand and the decision to have the operation must be entirely hers. Usually, however, patients with detrusor dyssynergia have been so

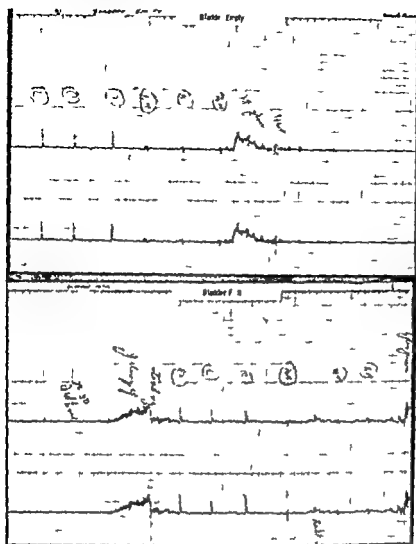


Fig 6 Detrusor dyssynergia Type I and II 10 days post-operative to partial resection of infravesical nerves bilateral (same patient as Fig 5). This operation was performed in 1976. She has excellent normal bladder control since the absence of abnormal contraction.

miserable for so many years that they are willing to risk the chance of failure.

OPERATION

1 A circumferential horseshoe shaped incision as recommended by Ingelman Sundberg is made through the mucosa of the anterior vaginal wall extending forward from each lateral vaginal fornix to join at a point about 4 cm posterior to the external urethral meatus (Fig 7).

2 The anterior vaginal mucosa is dissected free posteriorly to the level of the cervix.

3 The tissue lateral to the cervix between the vesical ligaments and the pubococcygeus muscles are dissected free in an antero-posterior direction by opening and closing long thin bladed tissue forceps or narrow bladed scissors.

4 Long narrow bladed retractors are inserted to the level of the cervix upward and lateral traction usually

discloses strands of whitish nerve fibers ascending and anastomosing with the inferior vesical artery. The combined nerve and artery bundle averages 4 to 5 mm in diameter (Fig 8).

5 With the artery nerve bundle exposed for a distance of about 3 cm clamps are applied leaving an interposed artery nerve bundle of about 2 cm. The interposed artery nerve bundle is excised and sent to the laboratory for nerve identification. We prefer to tie the clamped artery nerve bundle ends with polyglycolic acid ligatures no. 3/0 although Ingelman Sundberg used coagulation cautery.

6 If the procedure is to be performed bilaterally the opposite side is similarly dissected.

7 With the bleeding controlled the edges of the vaginal mucosa are approximated with continuous locking 3/0 polyglycolic acid suture.

8 Continuous bladder drainage is established by inserting a suprapubic cystostomy. Bonanno catheter. After 3 to 5 days the catheter is clamped and initiation of voiding is instituted.



Fig. 7 Curvilinear incision in the anterior vaginal mucosa the two posterior arms of the incision extending over lateral vaginal walls near the level of the cervix

Once voiding is well established usually by the 4th operative day the bladder tonus is again tested by a electronic urethrocystometry. A successful operation is indicated by complete suppression of all abnormal detrusor dyssynergia contractions both Types I and II.

RESULTS

Through Ingelman Sundberg employed the operation for various kinds of urinary incontinence including urgency incontinence infectious incontinence and enuresis he gradually restricted indications for the procedure to patients with uninhibited detrusor bladders.

At Henry Ford Hospital the procedure has been used only for patients disclosed to have severe detrusor dyssynergia Types I and II as evident on electronic urethrocystometry. Over the past 14 years 73 patients have been subjected to partial infravesical nerve resection. They ranged in age from 37 to 84 years with the average age 52. All patients but 3 have been followed for more than 1

year. In 4 patients the operation was a complete failure. In 4 patients substantial relief was obtained but in 25 patients urinary incontinence occurred if they

allowed their bladders to become excessively full or if they developed urinary tract infection. Two patients were apparently cured initially but after 1 year showed evidence of gradual recurrence of detrusor dysfunction of a relatively minor degree. In 1 patient there was an apparent immediate failure but after several months she gradually improved and presently has quite satisfactory urinary control. Incidentally this last mentioned patient was a teen aged daughter who has been shown to have detrusor dyssynergia Types I and II. The remaining 12 patients are apparently cured. Direct electronic urethrocystometric studies have indicated that of the 3 patients followed for less than one year 2 are cured and one is substantially improved.

CONCLUSIONS

Although partial infravesical nerve resection is an empiric operative procedure for patients with idiopathic detrusor dyssynergia it may be the only means of offering hope for a condition which is otherwise incurable. To Ingelman Sundberg must go credit for his genius in rationalizing the idea and for his skill in devising the surgical technique.

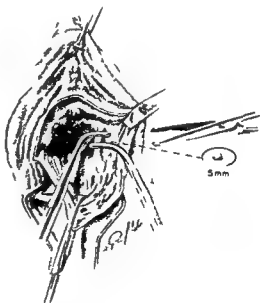


Fig. 8 Exposure of the artery nerve bundle lateral to the cervix. Each nerve bundle is doubly clamped leaving a 3 cm intervening segment. The nerve bundle is cut and the intervening segment sent to the laboratory for microscopic identification of nerve bundles.

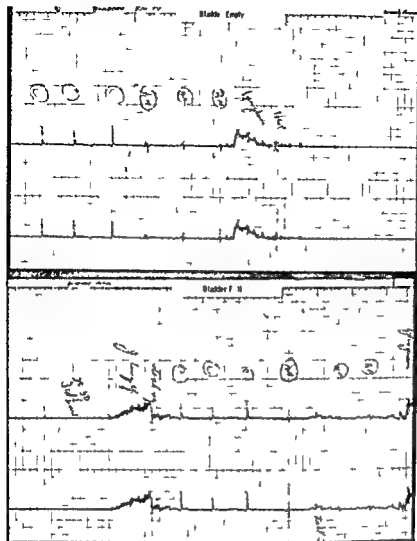


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RESULTS OF OPERATIVE TREATMENT OF STRESS URINARY INCONTINENCE WITH SPECIAL REFERENCE TO PREOPERATIVE CLINICAL AND RADIOLOGICAL EVALUATION

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Abstract Operative results of stress urinary incontinence discussed on the basis of a follow up study made 3-5 years after the operation. The series consisted of 301 patients operated on during 1969-1971. The most significant anamnestic feature was the great number of multiparas. 67% of the patients had given birth to at least 4 children. The choice of the operative method was based on Green's urethrocytographic classification into type I and type II. The healing was complete or satisfactory in 88% of the total material. In the group operated on according to type I the outcome was good in 88% while in type II group it was good in 84%. Incontinence of type I was cured in 89% of the cases while patients of type II in only 72%. If there was a significant urgency incontinence observable in the incontinence symptoms preoperatively a good outcome was only attained in 64%. In pure or dominant stress incontinence the corresponding cure was 88%. Hysterectomy combined to the operation seemed to improve the results. Age, parity, duration of the incontinence symptoms, weight and changes of weight after the operation had no significant effect on the healing. The authors emphasize the importance of careful preoperative diagnosis based on the anamnesis and examinations in choosing the therapeutic method for incontinent patients.

The etiology of stress urinary incontinence is attributed to weakened supportive structure of the pelvic floor which results in a disappearance of the obturator urethrovesical angle and a change in the direction of the urethral axis during straining. Edgerton (9) postulated stress incontinence as being mainly due to a descent of the proximal portion of the urethra into a more caudal position than normal whereupon it can no longer resist the increased hydraulic intravesical pressure. According to Hutch (10) the low position of the basal plate of the bladder leads to a partially open internal orifice

of the urethra which results in a decline in urethral resistance. As a consequence of these anatomical changes the even application of the intra abdominal pressure wave on the urethra is prevented. Intravesical pressure thus exceeds the intraurethral pressure and urine escapes upon straining (3).

In differential diagnostics stress incontinence should first of all be distinguished from urgency incontinence which frequently has a background of infections as well as mucosal atrophies. A psychosomatic and neurological background is also often noted. This type of incontinence should be corrected conservatively.

The results obtained by operative treatment of stress incontinence vary greatly. Using the vaginal operation (14, 15) the cure rates have been between 50-77% (13, 18). Ingelman Sundberg (11) pointed out that recidivous cases are common after the Kelly-Kennedy operation; the pubo-coccygeoplasty developed by him resulted in recovery in 94% of the cases and distinct alleviation of symptoms in 6%. The results obtained from suprapubic vesicourethral suspension (16) are variable from 65% to 98% (4, 6, 17).

The 266 patients with urinary incontinence operated at the Department of Obstetrics and Gynecology, University of Oulu during 1965-1967 were analyzed previously (12). A follow up examination made 3-5 years after the operation showed 57% patients to be fully asymptomatic and 28% to suffer from merely mild incontinence. In 1968 we adopted the method of Green (5) for choosing the operative technique on the basis of bead chain urethrocytography findings. Type I includes the

Table I Distribution according to age groups

<30		30-39		40-49		50-59		≥60	
N	%	N	%	N	%	N	%	N	%
5	2	42	14	116	38	110	37	28	9

patients in whom the posterior urethrovesical angle is straightened upon straining but the urethral inclination angle remains smaller than 45°. In type II the inclination angle becomes greater than 45°. The type I patients are operated on via the vaginal route (Kelly-Kennedy plastic) (14, 15). In type II suprapubic suspension of the vesical neck and the urethra is accomplished with the Marshall-Marchetti-Krantz operation (16). The value of anamnestic data in accurate preoperative differential diagnosis of urinary incontinence has been emphasized.

The purpose of this follow-up study is to present the results of the operative treatment of incontinence patients, taking into account the choice of the type of operation, the severity of incontinence and the preoperative nature of the incontinence.

MATERIAL AND METHODS

During the years 1969-1971, 301 patients with urinary incontinence were operated on at the Department of Obstetrics and Gynecology, University of Oulu. The mean age of the patients at the time of the operation was 48.4 ± 8.5 years (Table I) and 75% of them belonged to the age group of 40-59 years. The patients had had an average of 5.1 ± 2.9 deliveries before the operation (Table II). 67% of them had had 4 or more deliveries and 10% belonged to the group of 10 or more deliveries. The average duration of the symptoms of urinary incontinence before the operation was 7.9 ± 7.4 years. Concurrent uterovaginal prolapse was noted in 79 patients (26%). The mean weight at the time of operation was 68.4 ± 10 kg. 250 patients (83%) had an occupation requiring manual work. 150 (50%) had been delivered of at least one child weighing over 4 kg. 25 (8%) had had a vacuum extraction or forceps delivery. Recidivous urinary tract infections had been suffered preoperatively by 126 subjects (42%). The symptoms of 220 (73%) had appeared before menopause. 15 patients (5%) had previously been operated on because of urinary incontinence. The grading of incontinence is presented in Table III. 56% of the present series belonged to grades II or III. The anamnestic classification of the patients according to type of incontinence shows that 274 (91%) had pure stress incontinence or a mixed type of incontinence dominated by stress symptoms. 27 (9%) had urgency symptoms dominating over the stress symptoms. They were operated on because of concurrent uterovaginal prolapse or a failure of conservative treatment.

84 patients (27%) underwent a vaginal Kelly-Kennedy operation, sometimes combined with vaginal hysterectomy (Table IV). The Marshall-Marchetti-Krantz operation, either alone or in combination with various vaginal or abdominal measures, was used in Green's group II (72%). Three patients underwent a sling operation.

The average period between the operation and the follow-up examination was 47 ± 8 months. 9 patients (97%) participated in the follow-up personally, while 10 did so by a letter. 98 patients with continuing incontinence symptoms were subjected to cystometry in the follow-up examination.

RESULTS

The effect of the operative methods on the results

95% of the patients had become entirely free from incontinence and 30% had recovered enough not to have daily inconvenience (Table V). The overall percentage of recovery is hence 85%. The mean time between the operation and the appearance of recidivous symptoms of incontinence was 11.8 ± 11 months. The operation for Green's type I resulted in healing in 88% (Table V); the corresponding value for type II being 84%. Additional hysterectomy improved the results (Table VI). If the type of operation was a pure Marshall-Marchetti-Krantz suspension, the results were evaluated as cured or improved in only 64%. Combining the MMK operation with abdominal hysterectomy, the corresponding value was 66%. If the MMK operation was performed with vaginal raphes or vaginal hysterectomy, a good operative result was achieved in 86%. For 14 patients (5%) the outcome of the prolapse operation was nonsatisfactory. This had no effect on the healing of urinary incontinence.

The effect of other factors on the results

Urinary incontinence was healed in 89% of the patients with grade I incontinence, 84% of the grade II patients and only 72% of the grade III patients (Table VII). The differences are not significant ($p < 0.05$), however, 88% of the patients with pure or dominant stress incontinence recovered while only 64% of those with urgency symptoms.

Table II Distribution according to parity groups

0		1-3		4-6		7-9		≥10	
N	%	N	%	N	%	N	%	N	%
7	2	94	31	118	39	51	17	30	10

Table III Classification of urinary incontinence

Method	N	%
1 Urine leaks when coughing	132	44
1 Urine leaks when walking	145	48
1 Urine leaks even at lying	24	8

was dominating preoperatively did so (Table VIII) his difference is highly significant ($p < 0.001$)

The following factors on the other hand had no effect on the results: age at the time of the operation, parity, preoperative duration of urinary incontinence and weight at the time of the operation or change of weight after it.

Recurrent urinary infections had been suffered postoperatively by 104 patients (35%)

Comparison of pre and postoperative continence

This comparison also includes the cases where the incontinence was not completely cured by the operation, though it did not cause daily inconvenience (Table IX). 61% of the cases classified as pure stress incontinence preoperatively came symptomless but 13% developed incontinence dominated by urgency symptoms. In the cases where urgency symptoms dominated preoperatively a good operative outcome was obtained in 26% but 61% continued to have mild or severe urgency symptoms.

Results of postoperative cystometry

The findings of the 98 follow up examinations are presented in Table X where the results are compared according to the type of postoperative incontinence. Even those with mild postoperative incontinence are included. The bladder volume was be-

Table V Results of operative treatment by different methods

Postoperative course	Vaginal		Suprapubic		Total	
	N	%	N	%	N	%
Cured	71	88	185	84	166	55
Improved					90	30
No change	10	12	35	16	37	13
Worse					8	3

low 300 ml in 20% of the patients with urgency symptoms dominating the postoperative clinical picture. The corresponding proportion of patients with stress incontinence was only 4%. Increases of intravesical pressures exceeding 5 cm H₂O upon 300 ml filling were recorded for 65% of the patients with urgency incontinence. Pain on filling with less than 300 ml was more than twice as common in the subjects with urgency symptoms as in those with stress symptoms at the follow up examinations.

DISCUSSION

The present series was characterized by high parity values and severity of preoperative incontinence. Stress incontinence is mainly due to anatomical weakness of the muscle of the pelvic floor which is primarily attributable to pregnancies. The proportion of multiparas in series of stress incontinence has previously been found to vary within 95–97% (5, 12). The present series only included 7 nulliparas while 2/3 had 4 or more deliveries and nearly 1/3 had 7 or more. The parity rates do not however correlate with the recovery figures. The preoperative severity grade of urinary incontinence was estimated as II or III in 56% of the cases. This series hence represented more serious incontinence.

Table IV The operative methods used

Method	N	%
Vaginal raphies or Manchester+Kelly	58	19
Vaginal hysterectomy+Kelly	74	8
Marshall-Marchetti-Krantz (MMK)	48	16
Abdominal hysterectomy+MMK	48	16
Vaginal raphies+MMK	72	24
Vaginal raphies+abdominal hysterectomy+MMK	33	11
Vaginal hysterectomy+MMK	15	5
Sling-operation	3	1

Table VI Effect of the combination of hysterectomy with the incontinence operation on the results

	With hysterectomy		Without hysterectomy	
	N	%	N	%
Cured or improved	108	89	148	87
No change or worse	13	11	32	19

Table VII Effect of degree of urinary stress incontinence on the results

	Grade					
	I		II		III	
	N	%	N	%	N	%
Cured or improved	117	89	121	84	18	72
No change or worse	15	11	23	16	7	28

Table VIII Effect of the preoperative urgency symptoms on the recovery rate

	No urgency		Significant urgency	
	N	%	N	%
Cured or improved	230	88	25	64
No change or worse	31	12	14	36

Table IX Relations between pre and postoperative incontinence

Postoperative incontinence	Preoperative incontinence			
	Stress		Significant urgency	
	N	%	N	%
No incontinence	159	61	10	26
Stress	69	26	5	13
Significant urgency	34	13	24	61

than the previous series from the same clinic (12) the preoperative severity grade correlated with the outcome of operation although the differences were non significant

The present results agree with those presented by Green (7) as regards both the distribution of the patients into type I and type II and the operative results obtained in these groups. Only 77% of the present patients belonged to group I, 88% of them recovered well while 84% of the patients with suprapubic operation had an equally good outcome. The corresponding values reported by Green (7) were 95% and 91%. Bailey (7) and Green (7) have maintained that a vaginal operation leads to healing in only about 40% of the type II cases. Our results show also that operative results of vesicourethral suspension (Marshall-Marchetti-Krantz) can be improved by simultaneous raphies or hysterectomy which agrees with earlier observations (6). Combination of hysterectomy with the incontinence operation seemed to improve the results which agrees with the previous observations (7, 12).

According to the present findings the operation eliminated the symptoms of incontinence either entirely or to a clinically insignificant level in 85% of the cases which is in full agreement with our earlier results (17). Green's classification (1), which was adopted into use in our clinic, has had no improving effect compared with the previous series. Our results were not equally good as reported by the others: either Green (7) reported healing in 96%, McGuire et al (17) in 97%. That could be the reason for these differences. In the present series the preoperative differential diagnosis of incontinence had been based mainly on the anamnesis and no cystometric studies or cystoscopy had been performed systematically. In the series of McGuire et al (17) the operation had been preceded by careful urodynamic investigations. The present series included 9% of patients diagnosed to have dominantly urgency incontinence.

Table X Relation between the nature of postoperative incontinence and the some cystometric findings (96 cystometric examinations)

	Volume of urinary bladder				Increase of intravesical pressure at 300 ml				Bladder volume at the appearance of pain			
	≥300 ml		<300 ml		<5 H ₂ O cm		≥5 H ₂ O cm		≥300 ml or no pain		<300 ml	
	N	%	N	%	N	%	N	%	N	%	N	%
Stress	50	96	2	4	32	67	70	38	40	77	17	33
Significant urgency	17	80	9	70	16	35	30	65	22	48	24	57

operatively. Only 64% of these had a good outcome which impaired the results of the total series. 7% of the cases with pure or dominant stress incontinence had a good outcome. This difference is highly significant ($p < 0.001$). On the other hand 13% of those with dominantly stress symptoms preoperatively turned out to have a dominance of urgency symptoms after the operation. The reason for this may be that the preoperative anamnesis did not reveal the urgency symptoms which only appeared after the stress incontinence had been corrected operatively. The irritation of the detrusor muscle or the urethra by the operation or a high frequency (35%) of postoperative recidiv urinary infections may also contribute to this. The results here obtained emphasize the great need to be taken in the preoperative differential diagnosis of incontinence patients. Our clinic has in the last few years introduced a questionnaire which enables to differentiate accurately between the different types of incontinence. Routine cystoscopy and cystometric studies have also been taken in operative praxis. Special attention should be directed to the detrusor dyssynergy symptom (1-8) as this type of incontinence is regarded as representing the form of urinary incontinence second in frequency. It is due to involuntary activity of the detrusor muscle which is characterized by appearance of incontinence a short time after the elevation of intra abdominal pressure. This type of incontinence which should be treated conservatively may be confused with stress incontinence. We intend to continue using the current operative methods in the future but will try to render the preoperative differential diagnosis of incontinence ever more reliable.

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PREREQUISITES FOR TUBOPLASTY

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Techniques in measuring tubal patency and interpretation of findings can be misleading. Laparoscopic examination of infertile women with suspected diseased fallopian tubes is always prerequisite to tuboplasty. Operations must be accompanied by intrauterine injection of adequate amounts of dye and gentle tubal manipulation. Determined efforts should be made to eliminate destructive operations on nonsalvageable tubes before successful term pregnancies following tuboplasties are related more to the extent of tubal damage than to operative technique or postoperative management.

Many infertile women with diseased Fallopian tubes do not have tuboplasty because of contraindications existing such as age, a history of pelvic tuberculosis, obesity or a recent adnexal infection. With residual pelvic tenderness. Infertility studies may reveal seminal inadequacy, poor sperm motility or ovular dysfunction. When the limited chances of success are described, some women refuse operations. In a report by Murray (1) on 2 148 infertile women, 311 (14.5%) were operated upon and of these 86 had tuboplasty. Crane & Wood (2) reporting their experiences with 96 tuboplasties maintained that the properly selected patient and extent of peritubal adhesive disease greatly influenced prognosis. O'Brien and colleagues (3) reviewed 173 tuboplastic operations and of them on private patients followed by a 35% pregnancy rate. Of 1 126 clinic patients seen during a 10-year period for infertility, 424 (37%) had tubal disease but only 117 came for reconstructive surgery.

The incidence of tubal disease reported as a cause of sterility in any series varies with the type of patients studied. Peterson & Behrman (4) noted that 5% of women with unexplained infertility had subsequent tubal pathology at laparoscopy. Corson & Bologna (5) found tubal disease in 145 (58%)

of 249 patients undergoing laparoscopy for infertility.

The results from conventional nonoperative tests for tubal patency, uterotubal insufflation and hysterosalpingography, may be misleading because of the inherent limitations of these procedures. The results also depend upon the physician's technique and interpretation. A presumptive diagnosis of tubal occlusion during insufflation can be made if passage of carbon dioxide does not occur below 200 mmHg, kymographic oscillations are absent and no shoulder pain follows the test. Of 500 infertile women who eventually became pregnant, Rubin (6) noted initial pressures of 200 mmHg on one or two occasions in only 12 patients (2.4%) but none conceived after three negative tests. In a similar group of 296 infertile women, Siegler (7) found only one patient who conceived after three negative tests. These observations suggest that reported failure of carbon dioxide to pass through Fallopian tubes is indeed a poor prognostic sign. Uterotubal insufflation is not too accurate in the presence of dilated, occluded ampullae resulting occasionally in misleading or false positive tests.

Salpingography can show the size of a distal tubal obstruction but not the condition of fimbriae, the degree of tubal fixation or endosalpingeal destruction. Persistent localized collections observed on the follow-up X-ray film suggest ampullary disease. Boyd & Holt (8) made a diagnosis of hydrosalpinx by hysterosalpingography in 130 instances and a subsequent laparotomy disclosed only four normal (3.1%) patent tubes. Hysterosalpingographic interpretations of distal obstruction without dilatation is less accurate because at laparoscopy many tubes show patency but are restricted by peritubal adhesions. Young and colleagues (9) per-

formed tuboplasties on 112 infertile clinic patients confirming the observations of Özaras (10) who correlated postoperative pregnancy rates following salpingoneostomy with preoperative radiologic rugal markings. Their presence indicated minimal endosalpingeal disease. 60% of the patients conceiving after tuboplasty while in their absence only 7% became pregnant.

Swolin & Rozencrantz (11) compared findings of laparoscopy and hysterosalpingography in 143 patients, both methods being in agreement in the diagnosis of partial or complete tubal obstruction and tubal adhesions in 90 women (63%). Significant tubal abnormalities differing in degree were seen with both techniques in 24 other patients (17%). Normal laparoscopic findings were found in 14 women whose hysterosalpingograms were interpreted as abnormal. Bilateral tubal patency was detected in a previous hysterosalpingogram in 12 patients although partial obstruction was noted on laparoscopy. Radiologic studies were performed before ovulation with water soluble media followed the next day by laparoscopy using methylene blue or indigo carmine with saline. Maathuis and colleagues (12) and Coltart (13) found in comparing the results of hysterosalpingography and laparoscopy that most errors occurred because detection of significant peritubal adhesions cannot be made by radiologic techniques. Results from insufflation and salpingography should be carefully interpreted but it is sometimes difficult to evaluate the extent of tubal abnormalities without endoscopy. Although Frangenheim (14) who no longer advocates hysterosalpingography in infertile women he does advise laparoscopy to evaluate the pelvis and has recommended tuboplasty in almost one third of his patients.

In bilateral cornual blockage contrast fluid does not fill the tubes and none is seen in the peritoneal cavity on the delayed film. In these patients it is important to record the amount of fluid used, the degree of leakage at the cervix, the force and resistance to the instillation and the patients' complaint of pain. Careful search for intramural and isthmic filling is important because their demonstration can influence the selection of the type of tubal reconstruction. With laparoscopy the tubal isthmus can be palpated to search for the characteristic nodularity of salpingitis isthmica nodosa, fimbriae can be manipulated and observed, ostia can be probed and tubal patency can be tested by injecting dilute solu-

tions of dye through the cervix. Steptoe (15) advocates laparoscopy in infertile patients to verify patency with hydrotubation to assess tubal function and fimbrial involvement to evaluate the need for possible tuboplasty and to lyse peritubal adhesions. Operative descriptions are dictated routinely following the procedure according to an organized plan so that important information is not forgotten. Rarely endoscopic observations are misleading or difficult to interpret and an occasional unnecessary laparotomy is done.

Congenital or inflammatory luminal fibrosis or salpingitis isthmica nodosa are the most common causes for organic proximal obstruction. The condition is suspected if 1) nonpatency to CO₂ insufflation occurs at 200 mmHg for 2 min at a flow rate of 30 ml per minute with increasing suprapubic discomfort but no subsequent shoulder pain; 2) opacification of no more than 2 to 3 cm of the fallopian tube is seen after adequate amounts of contrast medium have been injected without subsequent distension; 3) at laparoscopy occasional blanching of the isthmic segment is seen with resistance to the injection of dye through the cervical cannula and some vaginal reflux. Tuboplasty is feasible if distal segments including fimbriae are normal. Isthmic filling of indigo carmine in grossly normal tubes without distension usually indicates insufficient dye rather than tubal blockage. The exact site of occasional isthmic area may be difficult to locate but occasionally isthmic distention and blanching are seen proximally to the obstruction. Salpingitis isthmica nodosa is missed on hysterosalpingography and laparoscopy unless careful observations are made. Palpation and manipulation of the tubal isthmus with a probe are essential. Laparoscopy is a prerequisite for tuboplasty in patients who have cornual or tubal blockage on radiologic studies because the condition of the distal tubal segments is unknown. Laparotomy intrauterine fundal injections of dye are attempted to prove obstruction before tubal resection and anastomosis or implantation. Occasionally this maneuver causes tubal filling despite previous studies indicating cornual obstruction. Even with final corroboration showing bilateral occlusion subsequent examinations of excised segments may not reveal any abnormality. Boyd & Holt (8) reported 34% of tubal from such patients examined histologically were normal.

In a patient desirous of reversing a tubal ster-

operation laparoscopy can verify the presence of distal tubal segment evaluate the gross appearance of the fimbriated end and enable the gynecologist to ascertain the feasibility of tubal reconstruction

referred increasing lower quadrant pains during insufflation suggest ampullary obstruction

degree of distension and character of mucosal folds can be seen on hysterosalpingography large proximal dilatations without luminal markings being poor prognostic sign Ozaras (10) found that only 8 patients who had slight dilatation and linear mucosal markings conceived after ampullary salpingostomy If ampullary disease is associated with salpingitis isthmica nodosa tuboplasty is contraindicated Rigid luminal contours indicate severe advanced salpingitis with fibrosis possibly suggestive of tuberculous salpingitis

laparoscopy in premenstrual phimosi caused by endosalpingeal ampullary adhesions free fimbriae are noted with tubal filling some dilatation or minimal spill of dye In the absence of peritoneal adhesions these problems can be treated by tubotubation In some patients in whom pregnancy still does not occur following ovarian wedge resection despite successful correction of anovulation laparoscopic examination is indicated to check for the extent of postoperative peritubal adhesions Relatively normal fimbriae can be observed by adhesions covering the adnexa and posterior leaf of the broad ligament Gentle manipulation probing and cervical instillation of dilute methylene blue can show tubal patency with adhesions or distal tubal filling without spill

laparoscopy chromotubation is indispensable especially if terminal tubal segments are fixed firmly in the cul-de-sac Tubal filling with only moderate distension but some spilling of dye in the cul-de-sac or behind occlusive adhesions is a favorable sign A dilated tube with pale thin walls distended with fluid before instillation of any dye is discerned as abnormal Additional pathology such as myomas or endometriosis indicates a poor prognosis

in diseased club-shaped distally occluded tubes have a more natural pink color feel less tense during probing and fill but do not spill fluid under perioscopic control An occasional hydrosalpinx fills with dye during chromotubation rupture

and extravasate material into the mesosalpinx thus explaining abnormal patency to CO₂ or contrast medium in some of these patients

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ADVANCES IN TUBOPLASTY

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Abstract Reconstructive oviductal surgery was performed on 105 private patients with primary and secondary infertility who were selected from 1075 endoscopic examinations. Each patient underwent an infertility surgery which included gamete formation, reception and deposition of gametes, nidation, post-coital and semen analysis. Only those with tubal abnormalities not responding to conservative therapy after a minimum period of six months following laparoscopic examination were selected for tuboplasty. Spiral stents for fimbrioplasty and eight teflon tubing for mid portion and cornua obstruction were employed. These stents were removed eight to 12 months post surgery under local anesthesia at the office. Of the 105 tuboplasties, 193 patients had sustained pregnancy, 75 conceived, 7 aborted and 1 had an ectopic pregnancy. Pregnancy occurred between 1 and 26 months after removal of the stents. Complications were very few. The use of Roland spiral teflon stents has resulted in a 100% increase in patency and pregnancy rates as compared to those without use of stents.

ADVANCES IN TUBOPLASTY

Experience accumulated in the field of infertility demonstrates abnormal tubal factors play a role in at least one third of all cases and are second only to the male factor in incidence. Oviductal reconstructive surgery for the restoration of fertility has gained increasing success as experience has been gained. Current improvements are based on the discriminating selection of patients by endoscopic and modifications of established techniques. The clinical evaluation of certain aspects of technique designed to improve ovum pick up, facilitate gamete transportation and expedite zygote passage through the tube is the subject of this report. Systems for properly selecting candidates for tuboplasty and for reporting the surgery and its results are also included in this study.

MATERIAL AND METHODS

This presentation is based on the results in reconstructive oviductal surgery in 105 patients from our private practice. These cases were selected from 1075 endoscopic examinations (48 culdoscopies and 1027 laparoscopies) in patients with primary or secondary infertility. Prior to endoscopic examination all patients were evaluated by either our staff or their own gynecologists.

Before we subjected any patient to endoscopic examination, each couple underwent the following office procedures:

Gamete Formation. Determined by endometrial biopsy, serum progesterone and basal body temperature charts. If any of these factors was negative, it was repeated in order to establish the positive results.

Reception and Deposition of Gametes. By means of semen analysis, post-coital degree of fern phenomenon and semen penetration tests, combination of hydrotubation followed by carbon dioxide insufflation (1) and when indicated, hysterosalpingogram.

Preparation for Nidation. By means of endometrial biopsy, serum progesterone (RIA) and basal body temperature charts. This is done premenstrually.

Upon the completion of the above procedures and if there existed either the tubal factor or possible ovarian pathology such as Steinoid ovaries, and if the latter failed to respond to several months of Clomid therapy, these patients should they fail to conceive within a period of six months following the infertility survey and therapy, would be subjected to endoscopy. Some of these couples had been worked up by other qualified gynecologists and if the survey included all the above mentioned tests and proper therapy used as stated above, certified by the abstract received, these patients would be admitted to the hospital for endoscopy. Most of these patients had laparoscopy and some, if indicated, also had hysteroscopy at the same sitting. It must be pointed out that almost all patients had tried to conceive for periods over two years. The only exception would be the elderly nulliparous patient (over 30 years of age) who would be subjected to endoscopy at an earlier date for obvious reasons.

Prior to use of endoscopy or stents in a group of 3 patients, the postoperative patency result of tubal surgery

Table I Distribution of results in patients subjected to endoscopy and found to have tubal pathology

Total number of endoscopies	1 075
Patients with no visible tubal disorders	699
Patients with disorders amenable to correction	317
Patients with contra indications to tuboplasty	39
Patients who refused tubal surgery	14
Patients who conceived after laparoscopy but without tubal surgery (within 6 months)	118
Patients in whom tuboplasty was performed	705

was only 46% and only 12% achieved pregnancies following tuboplasty without stents. Only 6% resulted in live births.

Table I illustrates the subsequent course of 376 of the 1075 patients subjected to endoscopy with presumptive diagnosis of tubal pathology established by the basic evaluation and confirmed by this procedure. It is interesting to note that 699 patients of the total endoscopies performed did not reveal tubal pathology to warrant surgery.

Table II demonstrates the results of tuboplasty after we adopted the routine use of endoscopy and splinting techniques.

Table III lists the time interval between tuboplasty and resulting pregnancy.

TUBAL RECONSTRUCTIVE TECHNIQUE

The reanastomosis of previously ligated tubes or midportion obstruction was performed over a straight teflon splint of narrow calibre with the simple approximation of the freshly transected debrided ends by 5-0 chromic interrupted sutures through the seromuscular coat (Fig. 1) (Fig. 1).

Cornual implantation of proximally occluded tubes is carried out by a significant modification of the standard procedure which has proven advantageous. The conventional technique creates a large opening with a cork borer and employs a fistula incision at the cornual end of the oviduct. This implantation which is fixed by sutures in the endometrial cavity is followed by healing with the endosalpingeal tissue growing in the cavity of the uterus. The large cornual opening in the myometrium and the tubal tissue in the endometrial cavity have proven unphysiological and in some cases have met with disastrous cornual distention and alarming bleeding at tubal abortion with tubal rupture. In contrast we employ a narrow trocar and cannula to establish a uniformly narrow cornual interstitial tract to the endometrial cavity. The management of the diseased portion of the tube is also critical. It is our concept that the maximum length of the tube must be preserved for physiologic transport of the zygote. Therefore to avoid sacrificing an undesirable length of tube we employ a wire probe and simply establish a channel in the proximal portion of the oviduct (?)

creating a new lumen. In addition we denude one centimeter of the serosa of the proximal portion of the tube. The straight teflon splint is then introduced through the fimbrial end of the oviduct and on exiting the cornual end is fixed within the endometrial cavity with two 4-0 chromic sutures on a large curved or straight Keith needle passed obliquely through the cavity and out the serosa of the fundus. The serosa of the oviduct is then approximated to the serosa of the fundus at the cornual insertion with interrupted 5-0 chromic sutures (Fig. 2-4). We believe the endosalpingeal epithelium grows over the splint from the tubal side but does not reach the endometrial cavity whose epithelium comes to line a part of the distal portion of the splint. We believe this to establish a more physiological union of the two epithelia at a more appropriate junction thus eliminating the impairment of the progression of the blastocyst through the contracted interstitial portion of the tube and permits timely nidation.

Fimbrioplasty which is most commonly called for has been accomplished with dramatic improvement in our results by the introduction of the Roland spiral teflon stent as has been previously reported (4, 6). The spiral portion of the stent has a cone shaped flare from the straight portion of the tubing so that it conforms to the shape of the ampullary and fimbriated portion of the oviduct. The teflon tubing is strengthened by a matrix of straight fine copper wiring which is malleable and assumes as well the shape of the spiral cone. The critical features of successful fimbrioplasty include lysis of all adhesions and adequate dilatation of the constricted or occluded abdominal portion of the oviduct with graduated teflon cones at times maintaining moistness of the fimbria with adequate irrigation. The spiral cone is then inserted in the ampulla with a grooved metal cone shaped guide and is fixed with three or four 4-0 chromic sutures in the serosal coat of the oviduct just proximal to and parallel with the abdominal ostium and tying these over the distal end of the spiral cone seated deeply in the ampulla (Fig. 5).

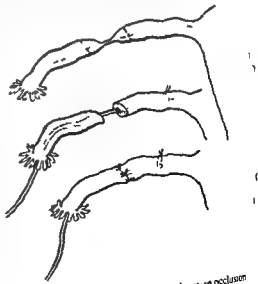


Fig. 1 Steps in reconstruction of midportion occlusion.

Table II Tuboplasty results in patients in whom pre operative endoscopy and stents were used

Type of lesion	Total no of patients	Post operative patency		Pregnancy		Abortion ^b No	Live births ^a	
		No	%	No	%		No	%
Laterally homogeneous Midportion	4	3	75	3	100		3	100
A For reanastomosis	11							
B Midportion occlusion	2							
Cornual	27	20	91	12	60	1	11	92
Fimbrial	135	129	96	45	35	4	41	91
Laterally mixed	44	41	93	15	37	7	13	87
Distal and "	205	193	94	75	39	7	68	91

^a pregnancies of patent tubes
^b of pregnancies

In all of these procedures employing teflon splints the tubal ends are each individually passed 2 cm apart through the layers of the abdomen to exit just beneath the skin. The two free ends are tied together in three well knotted easily palpable knots and fixed between the layers of fascia and the skin closure (Fig. 6). Eight weeks post-operatively is the appropriate time for removal. It is readily accomplished with minimum discomfort by observing the following rules. With local anesthetic infiltration a one centimeter incision is made just above the primary Pfannenstiel skin incision; the knot grasped and each limb individually tracted until recovered from the abdominal cavity. This gentle steady traction on one limb at a time straightens out the spiral cone and permits removal of the tubing. This simple office technique for removal of the stents replaces the formidable burden of a second operation to remove devices.

DISCUSSION

The proper work up of the infertile couple has been reported previously (7). An essential feature of the basic study is fiberoptic laparoscopy after gamete

Table III Time interval between tuboplasty and pregnancy

	No	Time interval in month				
		1-3	3-6	7-10	11-18	19-26
Midportion						
Reanastomosis of previous ligation	3		1			
Anastomosis of occlusion			7			
Cornual implantation	12		2	6	3	1
Fimbria						
Bilaterally homogeneous	45	1	5	12	18	9
Bilaterally mixed	15		1	4	8	2

formation in male and female reception deposition of gamete nidation and transport factors have been evaluated. So valuable is the information provided at laparoscopy that only in the most unusual circumstance is one justified in attempting tuboplasty without prior laparoscopy. In the primary infertility survey for a couple more simple techniques for estimating tubal patency and tubal configuration may be employed initially. These include hydrotubation followed by carbon dioxide insufflation (1) which must be carried out several times if an obstructive pattern is to be entertained. Also the volume of fluid introduced must exceed the routine ten ml if the presence of a hydrosalpinx with distal occlusion is to be discovered. Hysterosalpingography if an entirely physiologic configuration of the oviduct is demonstrated and accompanied by a good peritoneal smear bilaterally may be accepted in the initial survey and management of a couple. Since the incidence of adverse tubal factors is at least thirty five to forty per cent and since hysterosalpingography is deficient or in error at least twenty five to thirty percent of the time (6) and laparoscopy has proven therapeutic in at least twenty percent of cases with partial tubal fimbrial occlusion its use is warranted. In the large number of couples referred to the infertility specialist's office who have had previous infertility studies performed and have failed to conceive despite active supervision over six months laparoscopy is mandated an essential feature of the basic study and management.

A specific finding at laparoscopy not achieved by any other mode of investigation is the demonstration of narrowed abdominal ostia of the oviducts referred to as phimosis. On tubal perfusion

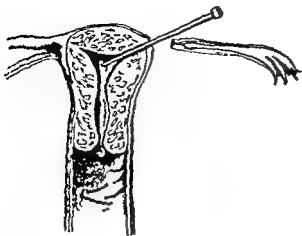


Fig 2 Opening at cornua made with a narrow trocar and cannula

under direct laparoscopic observation sacculation of the isthmic portion of the oviduct occurs and the perfused blue dye is prominently visualized through the serosal coat. This observation persists after the introductory pressure of the syringe for more than thirty seconds as one drop of dye escapes from the oviduct at a time. Another feature of laparoscopic observation in which hysterosalpingography frequently fails is the presence of peritubal adhesions at points along the length of the oviduct which may impede tubal motility and alter transport of the zygote. Also the presence of fimbrial

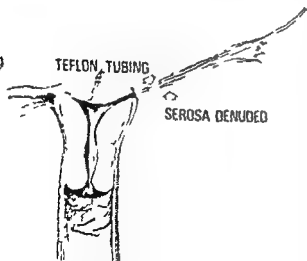


Fig 3 Teflon tubing threaded through the oviduct brought into uterine cavity with a 4-0 chromic suture and anchored to fundus

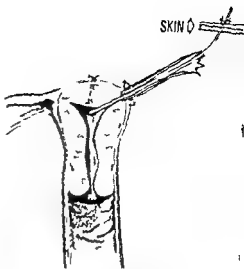


Fig 4 Interrupted sutures (4-0 chromic) used to approximate the serosa between the cornua and proximal portion of tube

adhesions which may impair ovum pick up readily fails to alter the peritoneal smear of hysterosalpingography (Fig 6).

To define the role of laparoscopy completely it must be insisted upon that tuboplasty must not be accompanied by endoscopy at the same time, especially with partial tubal occlusion. First, the therapeutic benefit of laparoscopy is significant and high as shown in Table II. The judgment of the role and prognosis of surgery is made hastily and under stress and not shared with the patient. Finally, the combined surgical procedure imposes too long an operating time which carries the burden of added morbidity in infection and a variety of complications. In our estimation only a previously inadequately described adverse laparoscopy supports warrants under unusual circumstances combined laparoscopy tuboplasty procedure.

The universal experience of a small but significant number of patients conceiving following hysterosalpingography and/or hysterosalpingography is not established. Nevertheless, our experience demonstrates an additional approximately twenty per cent of patients with tubal abnormalities who conceive after laparoscopy with no other definitive therapy (Table II). This is understandable on the basis that in the anesthetized patient under direct observation the oviduct may be exposed safely to significantly higher pressures at tubal perfusion so that gross material and fine synechiae may be easily

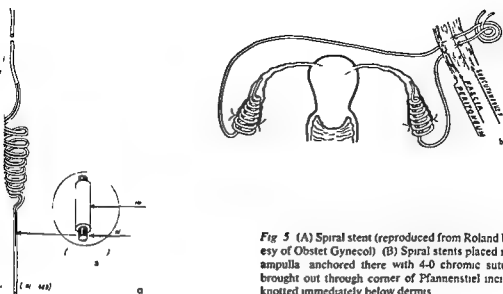


Fig 5 (A) Spiral stent (reproduced from Roland by courtesy of Obstet Gynecol) (B) Spiral stents placed into each ampulla anchored there with 4-0 chromic sutures and brought out through corner of Pfannenstiel incision and knotted immediately below dermis

This has been observed by us on numerous occasions at laparotomy for tubal plasty. We usually perform the oviducts through either the cannula at the cervical level or through the fundal area. In the latter we have observed the extrusion of this thick type grumous material. This can be further confirmed by the microscopic examination of the expelled material.

Current reports in the literature describe old and new techniques for lysis of tubo-ovarian adhesions and salpingostomy without the use of splinting devices and this in our opinion can only be deplored on physiologic grounds. Any manipulation that calls for a peritoneal reaction such as in dissection of adhesions or electrocoagulation of adhesions will militate in the establishment of new fibrous exudate as an essential step in healing. This must represent a defeat for the surgical technique. The subcutaneous fixation of the terminal knotted portion of the stents permits intestinal motion to move the knotted oviducts and inhibit formation of new fibrous adhesions and thus prevent adhesions between the distal portion of the oviduct to adjacent structures.

After laparoscopy those patients who were found to have partial tubal occlusion including those with hydrosalpinx partially occluded as demonstrated by persistent sacculations which do not disappear after thirty seconds were all followed by pre-ovulatory hydrotubation and carbon dioxide tubal insufflation described above for at

least six months. Most of them were followed for longer periods of time before they were recommended for tubal reconstruction. As stated elsewhere only those patients who were above 30 years of age and had a history of trying to conceive for a minimum of two years without success did we in cases of complete tubal occlusion as demonstrated at laparoscopy perform tubal surgery before the trial period of six months.

In cases of hydrosalpinx with distal occlusion



Fig 6 Adhesions between the fimbria and lateral wall of pelvis

we would simply incise the serosal covering and in most cases could observe a good amount of fimbria behind (as seen with $3\frac{1}{2}$ times magnification of ophthalmological lenses). In this case we would suture the serosal portion backward and still insert the spiral stent and anchor it there for eight weeks. Our rationale has been that by incising the serosal covering and handling the fine fimbria there usually exists the possibility to reform adhesions. When left alone the opposing walls usually collapse and are in approximation with each other. The insertion of the stent therefore is found to be very helpful in preventing re-adhesions between its walls. Prevention of the distal repaired tube from lying free and next to the abdominal contents might cause adhesions to the adjacent tissue. One must remember that by suturing the serosal area backward we are exposing raw surfaces to be subjected to re-adhesions. The stent in place and the distal portion of the teflon anchored keeps the abdominal wall distal portion from staying in one area and thereby kept moving by the intestine.

Our criteria for the spiral stent are two-fold: (1) to keep the reconstructed fimbria separated and thus prevent reocclusion; (2) in patients with phimosis and partial tubal occlusion after dilatation with graduated cone shaped probes the spiral stent prevents re-shrinking to its original position. The same holds true for the hydrosalpinx with occlusion by preventing the raw surfaces resulting from lyses of adhesions of the opposing walls to re-adhere. Our motto is—once adhesions have been lysed a stent must be placed.

Adjuvants to tuboplasty require evaluation at the same time. The role of corticoids and promethazine (Phenergan Wyeth Lab.) in inhibiting the formation of adhesions is not yet established (7). In pursuit of this information we are employing corticoids and promethazine in alternate cases. We are convinced that the prophylactic use of antibiotics is warranted in all cases of tuboplasty since an infectious factor in the etiology of tubo-ovarian adhesions can never be ruled out with certainty at the time of surgery. We therefore culture the distal portion of the tube at laparotomy. A further measure to eliminate the introduction of infection is the prohibition of sexual activity during the eight weeks period the stents remain in situ. We have recently added 200 cc of 8% Dextran in 5% Dextrose before closing the peritoneal cavity as additional aid to prevent post operative adhesion. So far it has served

a purpose. Oral contraceptives for eight weeks are provided to prohibit the conglomeration of healing epithelial surfaces by the menstruum and block cilia formation in the presence of semi-splinting material which might damage these functionally vital cellular elements.

Additional observations gathered from our years of experience with tuboplasty permit other pertinent conclusions to be drawn. And careful analysis of individual cases imparts to us a sense of awe and humility toward the work we do. In our series there were three cases that merit special complications characterized by intestinal obstruction, peritonitis and severe infection. Studied and contrasted there were features common to all three cases. All had severe pelvic endometriosis as the primary diagnosis. Two of these had previous surgery for pelvic endometriosis and had previous operative courses characterized by febrile episodes of clinical significance. The third an early case presented marked pelvic endometriosis requiring extensive dissection following which tuboplasty was performed with spiral teflon stents to protect the fimbria from re-involvement with adhesions. All three cases required laparotomy for intestinal obstruction and bowel resection. Most remarkable was the conclusion to the third case operated for tuboplasty for the first time. She conceived within a year and delivered a full term baby. Two caveats may be drawn from these cases. The presence of severe pelvic endometriosis presents a formidable problem the surgical management of which predisposes the patient to a stormy post-operative course frequently with superimposed infection. This combination of complications should preclude successful reconstructive tubal surgery. That even these complications may be overcome in the future as we acquire more experience and knowledge indicated by the third case which terminated in successful pregnancy.

An additional caveat derives from experience with micro-surgical techniques. We employ ophthalmological lenses providing three and one half times magnification and affording adequate visualization of all anatomic features especially the fimbriae kept moist with saline. Unlike neurosurgery the employment of microscopic techniques unnecessarily prolongs the surgical procedure and anesthesia time which may predispose to increased morbidity. We did not find more advantage in the rise to higher magnification

Table IV Proposed classification for reporting of up and management of reconstructive tubal surgery

- to be accepted for classification
- All other factors in reproduction have been demonstrated normal in both members of a couple
- The identical operation is performed in both tubes
- The status and management of the separately handled tubes are individually documented and described
- of the following data are reported
 - 1 Patient's prior obstetrical history and report of all infertility studies
 - 2 Fistula defect defined at endoscopy and peak pressures applied on tubal perfusion
 - 3 Operation performed
 - a Site, Fimbria and portion, cornual procedure
 - b Anures used
 - c Findings at tubal perfusion
 - d Type of stents used, duration of placement
 - e Operative diagnosis, pathological diagnosis of any resected tissue
 - 4 Postoperative management
 - a Hydrotubation
 - b Vasodilation
 - c Adjuvant medication
 - 5 Final technique
 - 6 Intraoperative performance
 - 7 Patency
 - 8 Obstetrical performance
 - a Abortions
 - b Live births
 - c Ectopic and location

SUMMARY

These results reflect a statistically significant improvement in the results of tubal reconstructive surgery. The more judicious selection of patients for tuboplasty by laparoscopy has significantly improved the prognosis for patients to whom surgical tubal reconstruction. It is important to observe in Table I the 39 patients in whom the pathological findings demonstrated tuboplasty was contraindicated. It must also be recognized that some of the conditions that interdicted surgery might not have been recognized on hysterosalpingography. Also it is reported that hysterosalpingography missed pathology in 30% of the cases. Therefore it may be said laparoscopy has permitted us to find additional cases to benefit

from tuboplasty over that number suggested by hysterosalpingography. These observations support our view that hysterosalpingography must not interdict early laparoscopic examination regardless of the findings. Further the therapeutic benefit of laparoscopy in a significant number of patients precludes the combining of the two procedures, laparoscopy and tuboplasty in most instances. Table I reveals 118 patients conceived following laparoscopy.

Table II reports the improved results afforded by the critical features of our technique, particularly the employment of the Roland spiral stent in fibrioplasty and splinting all anastomotic sites.

These tables reflect an improvement in oviductal patency rate from 56% to 90% when patients were more discriminately selected by laparoscopy and splinting with stents was employed routinely. The improved patency rate was accompanied by a concomitant rise in the pregnancy incidence to 39% of patients with sustained tubal patency.

The abortion rate is 3.4%. In groups the size of those reported here, these figures fail to demonstrate a predilection by site of tubal repair.

Table V Classification of tubal disease

	Right	Left	Bilateral
I Endometriosis			
A Ovary			
B Fimbria			
C Ampulla			
D Mid portion			
E Cornual			
F Cul-de sac			
II Malformation (occlusion)			
A Fimbrial			
1 Complete			
2 Partial			
3 With serosa only			
B Ampulla			
1 Hydrosalpinx			
2 Sacculization with dye only			
C Mid tube			
Complete occlusion			
D Cornual			
1 Complete occlusion			
2 Partial occlusion			
III Adhesions			
A Peritubal			
B Tube-lateral wall			
C Ovary-tube			

Table VI Classification of tubal surgery

Previous surgery Salpingectomy Oophorectomy
Salp Ooph

	Right	Left
I Peritoneal		
Excision of endometrosis		
A Ovary		
B Fimbria		
C Ampulla		
D Mid portion		
II Malformation (fimbrial)		
A Fimbrioplasty		
B Incision of serosa		
C Dilatation of phimosi		
D Insertion of stents		
type of stent hood spiral none		
III Mid portion occlusion		
A Excision of occluded portion		
B Anastomosis (end to-end)		
C Stent used (teflon poly)		
IV Peritubal adhesions Lysis (sharp or cauter)		
Kinks removed		
V Cornual implantation		
A Excision of occluded portion		
B Implantation (method)		
C Stent used (teflon poly)		
VI Adnexal adhesions Lysis		
A Between ovary-tube		
B Tube-lateral wall		
C Ovary-omentum		
D Omentum-tube		
E Cul-de sac (type-omentum endo-		
metrosis)		
F Endometrosis		

We have only one ectopic pregnancy to report so that no conclusions may be drawn in this regard

Finally of the 1075 laparoscopies 376 were found to have tubal pathology. The findings in 39 cases interdicted corrective surgery. 337 were amenable to reconstructive surgery. However 118 conceived after laparoscopy. 14 refused surgery. Therefore 205 patients had tuboplasty. 90% of these had sustained tubal patency. 68 had full term pregnancies. 7 had early abortions and may yet conceive and carry to term.

The implementation of finding tubal pathology with laparoscopy and the guidance it has provided in selecting cases for tuboplasty properly has been accompanied by additional improvement in our success with tuboplasty by the employment of rou-

tine splints in particular the introduction of a spiral teflon stent.

Further improvement we believe rests upon adoption of uniform simple classification of tubal operations so that statistically significant data can be accumulated. This will permit recognition of differences in the success rate met by the various techniques employed for tuboplasty. A meaningful system of classification should include a very limited number of subsets of procedures so that sufficient data for analysis will quickly accumulate. We would like to suggest the following criteria for a classification (Tables IV, V and VI).

Beyond the benefits to accrue from our analysis of our current techniques for oviductal reconstructive surgery further progress will be achieved as our knowledge of tubal function on a molecular level is increased. The role of hormonal activity in oviductal function in transport of gametes and in oogenesis is information which is being accrued. Functional sphincters and their hormonal control may be altered or restored by tubal plastic surgery. Tuboplasty and its adjuncts must preserve epithelial nutrition, the ciliary elements and the contractile motility of the oviduct so that sperm capacitation and timely ovum transport are insured. The molecular events must be better understood.

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THE LATZKO OPERATION FOR VESICO VAGINAL FISTULAE

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o-vaginal fistulae are seen infrequently in developed countries the main causes of operable being injuries due to hysterectomy radical rectomy or radiotherapy for carcinomas of cervix. The operative approach depends on factors such as the size and localization of the fistula, presence or absence of other lesions in the lower urinary tract or bowel. Various techniques and approaches have been proposed. For the uncomplicated vesico-vaginal fistula the approach preferred by gynecologists is the vaginal approach either the Fueth operation in German speaking countries or the SIMS procedure in the English speaking world. A good alternative especially in the posthysterectomy fistula but also in vesico-vaginal fistulae is the Latzko procedure.

Latzko (8) described this operation in 1913 and successfully in 3 cases. 1 with a recto-vaginal and 2 with vesico-vaginal fistulae. Independently Wert (9) and Fueth (10) have used the same method. Later Fueth (11) rediscovered it. The Latzko procedure has definite advantages over the Sims or the Fueth operation in cases of fistulae situated relatively inaccessible. The advantages are (12) a high success rate (100%) with little shortening of the vagina. The Latzko procedure may also be performed for radiation fistulae. In these cases the colostomy has to be more extensive.

various techniques—often a combined abdomino-vaginal approach—were chosen with or without the interposition of healthy tissue such as gracilis muscle (Ingelman Sundberg 6) bulbo cavernosus patch (Martius 10) or peritoneum (Hohenfellner et al 5). Of the uncomplicated 61 fistulae 23 were operated according to the Fueth or the Sims and 38 to the Latzko technique.

Time of operation It is generally accepted to postpone the operation for 6 or even 12 weeks and longer (7, 4, 7, 10, 13, 14) after the occurrence of a fistula and to wait 6 months or longer in cases of a radiation induced defect. If surgery is attempted earlier than 6 weeks the results are less favorable (1). During this interval a small postoperative fistula may close spontaneously under proper treatment. More important than the time factor is that any local irritation or bladder infection should be cleared before surgery is attempted.

The preoperative treatment is directed against local and bladder infection. Estrogens may promote healing in postmenopausal women. To exclude other lesions of the urinary tract a cystoscopy and an intravenous pyelogram or an isotope renogram should be made.

Technique The basic principle underlying the Latzko procedure is the observation that normally the vagina is collapsed the anterior wall lying on the posterior wall. A posthysterectomy vesico-vaginal fistula is always situated in the anterior wall. If the area surrounding the fistula is de-epithelialised the corresponding raw surfaces of the two walls may be approximated without any tension. It is important to remove only the thin epithelial layer and not the underlying connective tissue. Apart from the fact that the wound may always be closed without tension, Latzko's technique has three additional advantages (12): there is no danger of an ureteral damage during surgery, even a temporary postoperative overdistension of the bladder does not endanger the results, the repair is generally successful even after previous unsuccessful attempts.

If access is difficult the operation may be facilitated by making a median or mediolateral episiotomy and/or by pulling the fistula downwards with stay sutures. Allis clamps or a Foley catheter (Fig. 1) introduced into the fistula. If the defect is large enough the catheter may be introduced directly through the vagina into the bladder. If this is not possible a probe is passed through the urethra and through the fistula. A catheter is then fixed to the tip of the probe. By withdrawing the probe the catheter is pulled into the bladder. After filling the balloon the opera-

MATERIAL AND METHODS

Between 22 years 1953-July 1976 we have operated on 61 patients with vesico-vaginal fistulae. 61 uncomplicated and 4 complicated (combined with other defects of the urinary and/or intestinal tract). Most of these cases were referred from other institutions. In the complicated cases

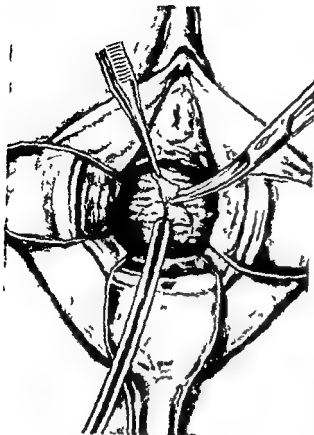


Fig 1 For explanation see text (Kåser in Matouschek & Kiermeier ref 11)

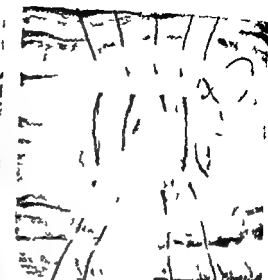


Fig 2 For explanation see text (Kåser in Matouschek & Kiermeier ref 11)

absorbable sutures 3-0 to 4-0 (Fig 2). More stitches are unnecessary and possibly harmful because of devitalisation. The vaginal mucosa is then closed either with a fine absorbable or non absorbable suture. The latter is removed after some weeks. The bladder is drained for 10-14 days via the urethral and/or suprapubic route. Lately we have been using the suprapubic method routinely because it is more comfortable for the patient and probably allows earlier resumption of spontaneous micturition.

tive field may be pulled towards the introitus of the vagina.

The fistula is now circumcized at a distance of 1 to 1.5 cm on the anterior and 1.5 to 2 cm on the posterior wall (Fig 1). The posterior dissection has to be more extensive because this wall has to cover the fistula. In order to facilitate the operation the circumcized area may be subdivided in 4 quadrants which are dissected successively. It is important to remove all epithelium from the circumcized area to the edge of the fistula opening. Generally there is little bleeding and no hemostasis except hot saline packing is necessary. An occasional bleeding vessel may be coagulated cautiously.

The wound is now closed in one or two layers with 4 to 5 interrupted sutures according to the size of the fistula and that of the vaginal fornix using atraumatic needles and



Fig 3 Schematic representation of the approximation of the bladder mucosa and the two layer closures (Kåser in Matouschek & Kiermeier Ref 11)

Table I Vesico-vaginal fistulae (Lat. Ao operation)

1st operation successful	35
2nd operation successful	2
3rd operation (combined abdomino-vaginal successful (radiological fistula)	1
Total	38

3 is a schematic representation of the different

ays 6 to 8 the catheter is clamped daily for a
of 2 hours and removed on days 7 to 10 if the
is able to empty her bladder. Micturition general-
ly presents remarkably little difficulty after suprapubic
c.

treatment Early ambulation presents no difficul-
ty. Treatment consists of antibacterial drugs, large
doses of fluid, daily bladder irrigation, supervision of
drainage and estrogens in postmenopausal pa-

tients. The patient may leave the hospital after 9 to 10 days or
if she so desires. She should be instructed how
to have intercourse before 8 to 10 weeks have
passed. Early intercourse has been the cause of failure in
published cases.

RESULTS

18 fistulae occurred after operative and/or
non-operative treatment for carcinoma of the cervix and
after hysterectomy—abdominal or vaginal—or
after resection of the cervical stump. In all but 3 cases the
operation resulted in cure. In two cases a sec-
ond operation was necessary and in 1 case only the
approach was successful. In many cases one
or even two unsuccessful attempts at
closing the fistula by various techniques had been
made before we saw the patient. The size of the
fistula varied from a barely visible pinpoint defect
to 2 to 3 cm in diameter (Table 1).
There are a few reports concerning the Latzko
operation in the literature with equally good results
(7, 9, 17). According to a paper from Graz
(Zal) and Erlangen (Germany) 29 out of 31
patients were cured by the Latzko operation (12).
In 12 patients had to be reoperated.

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INVERSION OF THE APPENDIX

There is nothing new under the sun

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Gynaecologists are trained to operate in the female pelvis and their expertise is in those organs of the abdomen lying below the umbilicus. They should have learned in their years of training to recognise the common variations from the normal in the peritoneal cavity. Diagnosis is the awareness of a condition, seeking and finding it.

This is not to say that gynaecologists should operate beyond their recognised realm which is the female genital tract, only to emphasise that it is the duty of anyone opening the abdomen to examine by eye and by the hand all the organs available for exploration and to note and make available for others information about abnormalities which may be found in the kidneys, gall bladder, liver and spleen. For instance, the presence or absence of Meckel's diverticulum should be recorded in the operation notes.

The vermiform appendix is the extra genital organ which most commonly intrudes into the gynaecologist's territory and it is tempting to remove it by appendicectomy (appendectomy) which involves cutting the base of the appendix from its continuity with the caecum, exposing the peritoneal cavity to the bacterial flora of the intestinal tract and burying the stump. In searching for a safer way of disposing of this useless and potentially dangerous organ, one realised after some years of thought that here was an organ, the blood supply of which could be divided easily and the whole appendage turned inside-out where it would necrose and fall or safely into its parent organ, the caecum.

A search of the literature showed that George M. Edebohl read a paper about this procedure before the Medical Society of the State of New York on February 4th 1895. Doctor Edebohl gives a full and reasoned account of the procedure and states

in view of the apparent simplicity of the conception, however, he should not be greatly surprised to learn that he had but furnished an additional illustration of the truth of the adage, 'nothing new under the sun'.

He goes on to say— the term 'inversion of the appendix' sufficiently explains itself. It consists in inverting into the lumen of the large intestine either the entire appendix or any part thereof remaining to the caecum, so that the appendix or what remains of it, instead of being free in the peritoneal cavity, is now free within the lumen of the bowel. The mucous lining of the appendix thus inverted becomes the external coat, and its former serous covering lines the new lumen, along the whole extent of which it lies in contact with itself. The mouth of the inverted appendix, on the peritoneal aspect of the caecum, is closed by suture to prevent re-inversion of the inverted appendix.

Inversion of the appendix should not be attempted in the case of acute appendicitis, because oedema of the appendix wall reduces mobility. Another contra-indication is narrowing of the lumen of the appendix in the proximal portions, for it must be emphasized that for successful inversion the most distal part must be able to pass within the proximal part of the appendix.

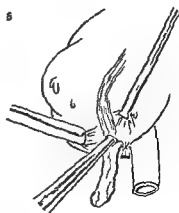
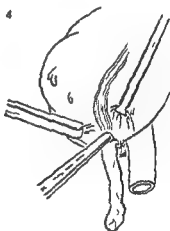
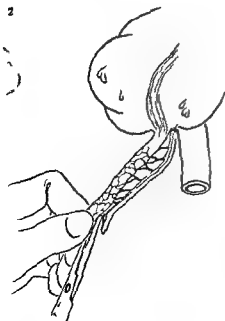
When demonstrating or describing this procedure, the inevitable question is asked: 'what happens to the inverted appendix?' When the main blood supply has been divided, the appendix is cast off and passes into the colon; if the division of the blood supply has been incomplete, the appendix may not slough, however, it will not cause an acute crisis, because the mucous surface is outward facing and inflammation does not occur within a confined space.

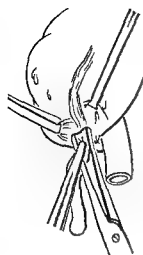
It is most important that the patient and her family doctor know what has happened to the appendix. They cannot be told that an appendicectomy (appendectomy) has been carried out. The most easily understood explanation is that the blood supply has been divided and the appendix turned inside out as when the finger of a glove is pushed into the palmar space.

The stages of the procedure for inversion of the appendix are best described by the following drawings:

1 A ligature has been passed to ligate the appendicular artery at the base of the appendix.

2 The meso-appendix is divided as closely as possible to the appendix.





3 All the contents of the appendix such as faeco-

4 The muscle wall of the caecum is picked up

5 The continuation of (4)

6 If there is difficulty in pushing the appendix

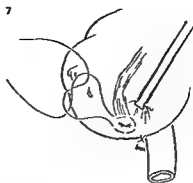
7 The appendix has been inverted into the cae-

8 and the peritoneal mouth is closed by a purse

9 string suture of chromic catgut no 00 and a further

10 purse string suture inserted to bury the first

7



ACKNOWLEDGEMENT

This non scientific and not original contribution is submitted as a practical reminder that there is nothing new under the sun. And in grateful thanks to Axel Ingelman Sundberg for his friendship and stimulating thoughts when we have met in many parts of the world.

I wish to thank Miss Patricia Archer Medical Illustrator at Guy's Hospital London England and her staff for the drawings.

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■ THERE A CHANGING EPIDEMIOLOGY OF PREMALIGNANT LESIONS OF THE CERVIX?

Results of Cytologic Screening of Pregnant Women

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Abstract The selection of women approached for cytologic screening is based on previous studies on age distribution and prevalence and consequent considerations on the risk of developing invasive carcinoma. In order to check if this is appropriate the results of the screening of pregnant women being selected irrespective of their age has been studied for two periods: 1961-64 and 1971-72. The frequency of lesions was remarkably high among the teenagers of both periods. A significant increase in the incidence of lesions was noted for the period 1971-72. This increase is most evident in the ages 21 to 30. A shift to more severe lesions was found among the teenagers and in the groups between 31 and 40. In conclusion more effort should be made with respect to cytologic screening of young women, particularly when pregnant. The increased frequency of cytologic atypias among women between 21 and 30 should focus the attention to the possibility that the epidemiology of premalignant lesions of the uterine cervix is changing.

The basis for screening of premalignant lesions of the cervix is found in studies on the prevalence and incidence of the lesion in different age groups and in different groups of the population considered at high risk. The interest and financial engagement of the society in screening programs differs considerably in different countries, even in different parts of the country as is the case in Sweden. Here the ages 20 to 50 are in focus, being checked every 3-5 years. In addition, pregnant women are being screened at the maternity welfare centers since the early sixties, irrespective of the age of the woman. The screening programs cover only part of the target population. Beyond comparison the best coverage is obtained within the maternity welfare system which serves a very high percentage of the pregnant women.

There is need for a reevaluation of the Swedish screening programs and if necessary to define new recommendations for the next decade, preferably uniform for the entire population of Sweden. The basis for the present screening programs was established several years ago and may have become inappropriate for two reasons. First, the interval between the cytological samplings may be too long; (2) second, the age groups at risk may have changed because of alterations in epidemiology. The second probability cannot be checked by study of attendants to the current screening programs or by study of the age distribution of premalignant lesions, as figures obtained in this way are based on selected age groups where women below 30 years of age are not represented to the same degree. The screening organization of pregnant women in Sweden instead has not taken into account the age of the woman and may therefore be used to check if there is reason to remodel the general screening program because of a changing epidemiology of the cervical lesions.

The present study compares two equally sized groups of pregnant women from around 1962 and 1972 with respect both to the relationship of incidence to age and to the distribution of various degrees of dysplasia and cancer in situ. The aim was to study if the frequency of premalignant lesions had changed in these groups of women during a ten year interval.

MATERIAL AND METHODS

A total of 4788 vaginal smears taken from attendants to the maternity welfare centers in the Stockholm area form

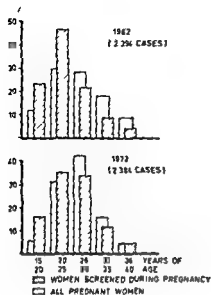


Fig. 1 Relative age distribution of screened and all pregnant women in Stockholm

the basis of the study 2394 consecutive vaginal smears from the period October 1961—September 1964 and 2384 from 1972 were studied. The interval between the two groups is thus approximately 10 years and the first period will in the following be briefly referred to as 1962. The age distribution in the two groups is shown in Fig. 1. Here the age distribution of the mothers taken from the birth statistics of the corresponding population is also shown. The figure shows for both periods an overrepresentation of smears taken from young pregnant women and an underrepresentation among the older women. In addition more smears were taken from young women in the earlier period. This may be due to neglectance of taking smears from women who recently had a smear taken for some other reason. There is reason to assume that such a neglectance was more frequent in the earlier period which would explain why a longer period was required in the early sixties than later to obtain the same number of smears. This dissimilarity of the two groups will be considered further in the discussion.

The cytologic slides were stained according to Papanicolaou and primarily examined by cytotechnologists. The cytologic findings were allotted to either of five diagnostic groups using the classification of Papanicolaou. All slides considered to contain cells of possibly premalignant or malignant significance were reviewed by cytopathologists being responsible for the final diagnosis which includes a verbal statement. In our screening routine usually a Pap III is equivalent to moderate dysplasia. Pap IV related to severe dysplasia or carcinoma in situ and Pap V implies a strong suspicion of invasive carcinoma.

To allow comparisons between materials collected several years apart the diagnostic criteria must have been practiced similarly during the two periods under study. This was tested by reviewing 190 consecutive positive smears from 1962 and 195 from 1972. The initial classifica-

Table 1 Revision of cytologic preparations for 1962 and 1972 by four different senior cytotechnologists

Cytotechnologist	Year	Papanicolaou		
		Same (%)	Higher (%)	Lower (%)
A	1962	67	9	24
	1972	64	16	20
B	1962	46	33	21
	1972	69	16	15
C	1962	57	28	15
	1972	68	18	14
D	1962	61	18	21
	1972	54	19	27
Average	1962	57	27	16
	1972	64	17	19

tion of these cases is given in Fig. 2. It is evident that material from 1962 shows a slight excess of more advanced changes as compared with that from 1972. A few cases in Pap I were included because of an initial filing which explains their presence in the group of positive cases. All these smears were now independently reviewed by four senior cytotechnologists.

This revision showed that there was no apparent difference between the evaluations of 1962 and 1972 (Table 1). The same result was reached in 60.5%. In most cases the difference in evaluation were restricted to one Pap group, i.e. a slide primarily allotted to e.g. group III was revised in 97% of the cases allotted to either group II, III or IV. Thus the criteria for the cytologic diagnosis have been practiced in the same way during the two periods studied.

RESULTS

Among the smears from the first period (1961-4) abnormal cytology was recorded in 135 specimens (5.6%) in contrast to 170 (7.1%) in the material from 1972 (Table II). This difference is statistically

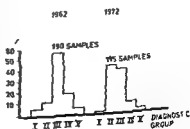


Fig. 2 Relative distribution with respect to diagnostic groups (Pap) of consecutive positive smears from 1962 and 1972

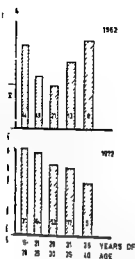


Fig 3 Frequency of squamous cell abnormalities (Pap III and IV) in maternity welfare patients from 1962 and 1972. The absolute numbers are shown inside the respective columns.

significant ($\chi^2=4.703$, $p<0.05$). The cytologic changes vary from mild dysplasia to carcinoma in situ. No case of invasive carcinoma was detected. The age distribution of all cytologic changes is shown in Fig 3. The difference between the two materials is notable, although the youngest group shows a high incidence at the same level in both. In the age group 21-25 the difference is statistically significant ($\chi^2=5.742$, $p<0.02$) and also in the age group 26-30 ($\chi^2=4.444$, $p<0.05$) with a higher incidence in the material from 1972. In the other age groups the differences are not statistically significant. If only the more significant atypias (Pap III and IV) are considered the difference between the two periods is similar (Fig 4). In addition the relative distribution of the different grades of cytologic atypia (dysplasia) have changed during the period studied. The proportion

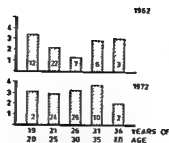


Fig 4 Frequency of more severe squamous cell abnormalities (Pap III and IV) in maternity welfare patients 1962 and 1972. The absolute numbers are shown inside the respective columns.

of Pap III and IV of all atypias has increased particularly among teen agers and in the group between 31 and 40 years of age (Fig 5). As only 4 cases with Pap IV were found 1962 and 5 in 1972 no conclusion can be drawn about the incidence of those cytologic atypias which indicate carcinoma in situ.

DISCUSSION

The problem of rational and economically acceptable screening of the population with respect to diseases of humanitarian and socio-economic significance has long been a main interest for governmental and other institutions concerned in the welfare of the population. The benefit of such screening programs to detect early cervical neoplasia has been demonstrated e.g. in British Columbia (1), Kentucky USA (2) and Østfold Norway (6). In Sweden a distinct reduction of the incidence of cancer of the cervix has been reported in those counties where screening programs were first initiated (5). For the whole country a moderate reduction of the incidence of cancer of the cervix has recently been reported (11).

The reported effect of the Swedish screening for

Table II Squamous cell abnormalities in maternal welfare patients 1962-1964 and 1972

	No of screened women	Women with squamous cell abnormalities	
		Number	Frequency (%)
1962-64	7394	135	5.6
1972	2384	170	7.1
Total	4778	305	

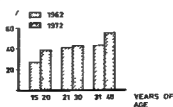


Fig 5 Proportion more severe squamous cell abnormalities (Pap III and IV) of all abnormalities (Pap II-IV).

cervical neoplasias are only moderate which may be due to inappropriate attention to the age groups at risk. Different sociological conditions are well known to influence the risk of developing carcinoma of the cervix. A reevaluation of the groups of the population being approached for screening may be necessary as these conditions may be changing.

The present material indicates that during the first period (1961-64) more women were examined cytologically because of clinical symptoms or findings or because of no recent cytologic examination. This would be expected to give a higher figure of the incidence of positive findings than is actually present in the age group in question. In spite of these circumstances the frequency of positive findings found is lower in the 1962 material than in the material from 1972. The real difference in this respect between the two groups must thus be still higher than indicated by the present figures.

Moreover, considering the severity of the lesions discovered, a change to more severe lesions is indicated by the obtained results (Figs 4 and 5) although the incidence of carcinoma *in situ* shows no change and no invasive cancers were found among our cases. As the clinical significance of mild cytologic atypias classified as Pap II may be limited, it is noteworthy that the differences between the two groups persist even when those cases presenting mild atypias are excluded (Figs 4 and 5). In this context it should be pointed out that cytologic screening during pregnancy is equally reliable for early cancer detection as in the non pregnant state (7-10). It is therefore generally recommended that pregnant women should be screened by means of exfoliative cytology for the purpose of cancer detection.

During the past ten years a considerable increase in the number of cases of carcinoma *in situ* reported to the Swedish cancer register has been a matter of concern (3). The increasing incidence among younger women may of course not represent a true change in the age distribution of the lesion but may be caused by increasing screening activities in the younger ages combined with a successive removal by treatment of asymptomatic cases in the upper age groups thanks to the screening program itself. Anyway, the recent figures of the age distribution of carcinoma *in situ* have been taken as an indication that an extension of the program to younger age groups is recommendable. This was also empha-

sized by Wallace & Slankard (9) who found carcinoma *in situ* in 12 of 7520 teenagers screened (0.16%).

However, the number of cases of carcinoma *in situ* reported in Sweden exceeds the number of invasive cancers by a factor of five in 1972, which was not the case a couple of years ago, a change in the practice of the diagnostic criteria may therefore be suspected which should lead to a fairly high number of diagnoses. These statistics are based upon the results of histopathological examination of surgical specimens, however. Our present study is based on examination of cytologic smears only. Revision of a number of slides from 1962 and 1972 excludes a change of opinion at our laboratory with respect to the diagnostic criteria (Table I). There is therefore good reason to consider the increased incidence of cytologic changes of possible premalignant significance as true.

In the two clinical materials reported here there is a remarkably high incidence of cytologic changes among the teen-agers. As teen-agers constitute a group where previous cytologic examinations are not common, this figure may be higher than the actual incidence in the age groups in question. Furthermore, the group of teen-agers reported here may not be representative for all teen-agers. Pregnancy in this age group may indicate a high sexual activity and an earlier sexual debut, possibly combined with a lower standard of contraception. This might also indicate lower socioeconomic and hygienic standards of living and perhaps also a higher degree of promiscuity, which all are factors known to increase the risk of cervical neoplasia.

The incidence of cytologic abnormalities (Pap III-IV) among the pregnant teen-agers of our study (in total 11 out of 621 or 1.8%) should be compared with the much lower incidence—0.17%—among non-selected teen-agers in Chicago recently reported by Fields et al (4).

Irrespective of the reason, the observed high frequency of lesions among young pregnant women in the present material defines them as a group of increased risk and strongly indicates that they should be included in cytologic screening programs. The high frequency of carcinoma *in situ* among teen-agers reported from Kansas City was given the same inference (9). In addition, both the increased frequency and severity of the lesions during the period studied indicates the necessity of expanding the general screening programs to younger women.

has been customary until now. The results also emphasize the importance of repeating retrospective studies of this kind at regular intervals in order to detect future changes in the epidemiology of early cervical neoplasia.

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EFFECT OF ALKOXYGLYCEROLS ON THE FREQUENCY OF INJURIES FOLLOWING RADIATION THERAPY FOR CARCINOMA OF THE UTERINE CERVIX

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Abstract: The incidence of injuries following intracavitary external radiation therapy is markedly decreased in all stages of the disease by the administration of alkoxyglycerols. Complex injuries (due to radiation injury and tumor growth in combination) were reduced to about 1/3 in group receiving alkoxyglycerols prophylactically, i.e. during and after radiation treatment, when compared with a control group. Using non prophylactic administration of alkoxyglycerols, i.e. during and after radiation treatment, no effect was observed on complex injuries while—as for the prophylactic group—the injuries due to radiation only were significantly decreased. The use of so called increased amount of radium in the secondary irradiator was followed by an unexpectedly high incidence of radiation injuries which was considerably reduced however by alkoxyglycerols especially when administered prophylactically.

Alkoxyglycerols are found in small quantities in several natural products. They are relatively abundant in the haemopoietic organs of mammals, particularly the bone marrow. These substances also occur in relatively high concentrations in the human mother's milk. They occur most abundantly however in the liver oil of certain species of shark. These oils contain up to 50% of alkoxyglycerol (1, 7, 8). The general formula for alkoxyglycerols is $\text{CH}_2\text{OH}-\text{CHOH}-\text{CH}_2\text{O}-\text{R}$ where R is a long-chain aliphatic radical.

The alkoxyglycerols have earlier proved to be of great interest. The first studies commenced with the oral administration of different fractions of cat bone marrow to cases of leukemia in children. Alkoxyglycerols promote the growth of *Lactobacillus* (1) and the formation of antibodies (2, 4). To some extent they prevent leucopenia and

thrombocytopenia as a result of radiation. The administration of alkoxyglycerols before, during and after radiotherapy for carcinoma of the uterine cervix also brings about higher survival rates than if radiation treatment alone is given (1, 2).

The aim with the present study has been to investigate whether or not alkoxyglycerols administered in a prophylactic or non prophylactic manner influence the incidence of radiation injuries and the growth of residual or recurrent tumour following radiotherapy for carcinoma of the uterine cervix. Problems connected with the development of radiation tissue damage following radiotherapy have earlier been elucidated in several publications from the Radiumhemmet in Stockholm where all patients included in the present study received their treatment (6, 9, 10, 11, 12, 13, 14).

MATERIALS AND METHODS

The clinical experiments in this study have been conducted using alkoxyglycerol preparations from the liver oil of the Greenland shark. The preparation produced by AB Astra with the working name AT III is a concentrate containing 85% free alkoxyglycerols. The content of different alkoxyglycerols from various sources is given in Table I.

The alkoxyglycerols were administered orally in capsules, 2 capsules 3 times a day, each capsule containing 0.1 g of alkoxyglycerols. The total daily dosage thus was 0.6 g.

The series of cases with invasive carcinoma of the uterine cervix treated at the Department of Gynecology Radiumhemmet, Stockholm, were reviewed during various periods. The patients comprised all stages, see Table II, and were allotted to one of the following groups:

Table I The percentage composition (weight) of alkoxyglycerols from various sources

Analyses according to Hallgren and Larsson (2, 3). The number of carbon atoms in the first column refers to the long chain component of the molecule. The number after the colon denotes the number of double bonds.

Alkoxy glycerols	Human bone marrow	Human milk	Liver oil Greenland shark
14:0			2.0
15:			0.7
16:0	29.4	23.9	9.1
16:1		trace	10.8
17:	7.6	3.6	3.6
18:0	24.6	22.8	2.8
18:1	16.7	33.8	59.4
18:2		1.4	1.6
18:3			?
19:	6.1	2.4	1.5
20:0	2.9	1.6	
20:1	3.2	2.3	6.2
22:0	0.7	0.7	
22:1	5.1	3.4	2.2
24:		2.1	

Both branched and normal chains C_{13} , C_{17} and C_{19} are present.

I. Patients given alkoxyglycerols prophylactically, i.e. during 7 days before, during the treatment period and for 1-3 months after the completion of therapy.

II. Patients given alkoxyglycerols only during the period of radiotherapy and for 1-3 months thereafter, non prophylactic administration.

III. Patients given radiotherapy only.

Groups I and II cases were treated during the period January 1, 1964-February 15, 1966. During this period 99% of the patients with carcinoma of the uterine cervix received alkoxyglycerols. Group III patients were treated during 1963 (348 patients) and February 16-December 31, 1966 (309 patients).

In addition to the patients followed up for more than five years and characterized above, a double blind study comprising 279 patients was conducted 1970-1972. These patients have been followed up for 3.5 years from the initiation of therapy.

The treatment was in almost all cases initiated with intracavitary radium application. This consisted typically of two radium insertions, separated by an interval of about three weeks. 53-200 mg of radium was homogeneously distributed in an intrauterine applicator, 19-70 mm in active length. The enclosure was 0.30 mm Au+0.35 mm Pt and a stainless steel container with a wall thickness of 2.00-2.75 mm. In cases in which both intrauterine and intravaginal radium was applied, no radium was introduced in the distal 15 mm of the cervix, whereas in cases of endocervical tumour growth, as well as in cases in which the growth had extended paracervically, the amount of radium in the intrauterine irradiator was increased (increased amount of radium) and radium sources were applied also in the low endocervix. No

radium was inserted in the vagina. The vaginal radium when employed was enclosed in a flat or curved applicator or in a few cases in two cylinders so maximum surface area and it was placed as close to the tumour as possible. The applicators were held in place by a tampon, which also increased the distance to the rectum. 58-250 mg of radium were used in the vagina.

The computation of doses delivered to the posterior wall of the bladder and to the anterior wall of the rectum was made on the basis of routinely performed dose rate measurements. Consecutive dose rate values were obtained at centimeter intervals progressing from the bladder by caudally through the urethra and along the rectum. Measurements were in all instances performed with a slightly modified Siemens Gammameter with the patient in lithotomy position immediately after the application of the intrauterine sources (9, 10, 15). The external radium therapy was given either with conventional roentgen or with cobalt irradiation according to one of the following techniques.

Four fields, two abdominal and two gluteal, were utilized when conventional roentgen rays were used. An exposure of 6x400 R was given over 4-6 weeks. External irradiation using Cobalt 60, two opposed beams was administered through 250-400 cm² anterior and posterior fields. An exposure of 7000-5000 R was given in fractions of 300-400 R six days a week. Lead shields were sometimes placed over the site of the uterine cervix in the anterior or the posterior beam or both in an attempt to

Table II Definitions of clinical stages*

Stage I	Carcinoma strictly confined to the cervix
Stage I A	Cases of early stromal invasion (pre clinical carcinoma)
Stage I B	All other cases of stage I
Stage II A	The carcinoma extends beyond the cervix but has not extended on to the pelvic wall
	The carcinoma involves the vagina but not the lower third
	No parametrial involvement
Stage II B	The carcinoma extends beyond the cervix but has not extended on to the pelvic wall
	The carcinoma involves the vagina but not the lower third
	Parametrial involvement
Stage III	The carcinoma has extended on to the pelvic wall. On rectal examination there is no cancer-free space between the tumour and the pelvic wall
	The tumour involves the lower third of the vagina
Stage IV	The carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum

* Definitions according to FIGO (International Federation of Gynecology and Obstetrics).

Table III Injuries following radiation therapy. All stages included

Abbreviations: R=injuries due to radiation treatment; C=complex injuries due to tumour growth or to a combination of tumour growth and radiation treatment; N₁=number of patients with mild injuries; N₂=number of patients with complex injuries; M=more than one injury per patient; multiple injuries.

Year	No of pts	I		R		C		N ₁		N ₂		M	
		No	%	No	%	No	%	No	%	No	%	No	%
1963-1966													
I	48	83	18.1	59	12.9	4	8.2	76	15.0	48	5	11	
II	381	103	24.4	34	8.9	59	15.5	62	16.3	41	10.8	3	6.0
III	617	244	37.1	150	22.8	94	14.3	129	20.9	6	11.6	43	6.5
1970-1977													
I	137	36	26.3	28	20.4	8	5.8	3	2.2	8	5.8	4	2.9
II	14	74	52.1	40	28.2	34	23.9	5	35.6	4	16.9	16	11.3

Administration of alkoxylglycerols *prophylactically* and during radiation treatment.

Administration of alkoxylglycerols *only* during radiation treatment.

Radiation treatment only.

these portions of the pelvis already having received a high dose of radiation from the intracavitary series. With a three beam technique Cobalt irradiation was delivered through one 250 cm² anterior field and two 500 cm² lateral fields. Weekly tumour doses were 700-800 rad with the total tumour dose being 4000-5000 rad. A blocking block was sometimes used in the anterior beam field or in part of the fractions. For data regarding the distribution of the dose from the external irradiation the factor is referred to previously published reports.

In the calculation of the incidence of radiation injuries (per rectum, ureters and intestine) the principles given by Kottmeier (14), Kottmeier & Gray (13) and Gray & Kottmeier (6) have been used in this investigation.

In earlier follow-up studies regard has been paid to injuries due to radiation treatment only (R). In this kind of study, however, it has been necessary to include the injuries due to tumour growth and to the combination of tumour tissue damage and residual or recurrent tumour growth. This is because the alkoxylglycerols may have a marked effect on the incidence of these injuries due to either tumour growth or to tumour growth and radiation damage. These injuries are called complex injuries (C). The sum of R and C is defined as the total number of injuries (I). Furthermore, in this study of the effects of alkoxylglycerols in connection with radiotherapy it has been considered necessary to give figures for the incidence of occurrence of more than one injury per patient, i.e. multiple injuries (M).

The injuries have been classified according to the following schedule given by Kottmeier:

Grade I Injuries producing mild subjective symptoms accompanied by minimal objective changes in the mucosa. These injuries are considered as radiation reactions and have consequently been omitted.

Grade II Injuries which are composed of moderately severe objective changes such as areas of necrosis, ulcers or moderate stenosis.

Grade III Bladder and ureter injuries comprising radia-

tion fistulas and rectal and intestinal injuries comprising stenoses that require colostomy.

Grade IV Rectal and intestinal injuries which are all fistulas.

Injuries which appear within three months of surgery plus radiotherapy have been excluded, and those injuries which are not clearly related to the radiation treatment or to tumour growth have also been omitted. Only the injuries which appear within 5 years after the onset of radiation treatment have been taken into consideration in this investigation. Patients with complex injuries (C) have clinically detectable cancer, residual cancer or recurrent tumour growth confirmed by biopsy or autopsy.

RESULTS

Reduction of injuries after administration of alkoxylglycerols

The effect of alkoxylglycerols on the incidence of injuries following radiation treatment has been studied comparing groups I and II with each other and with the control group III; the total number of injuries, injuries due to radiation treatment and complex injuries in the different groups are given in Table III A.

It is observed that

1 The incidence of injuries is considerably lower in the alkoxylglycerol groups than in the control group—especially for group I where alkoxylglycerols have been administered prophylactically. The incidence of total injuries is reduced to about 50%.

2 Complex injuries are reduced to about 1/3 in the prophylactic group compared with the control group.

Table VII Incidence of injuries associated with two different modalities of intracavitary treatment

Group	No of pats	I		R		C		N _I		N _C		M		3 year survivals		5 year survivals	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
A 1963-1966																	
<i>Intrauterine irradiator <100 mg Ra ek, vaginal irradiator <90 mg Ra ek at both treatment courses (L)</i>																	
I	224	38	17.0	29	13.0	9	4.0	36	16.1	7	3.1	2	0.9	190	84.8	16	7.6
II	178	32	18.0	14	7.9	18	10.1	23	12.9	12	6.7	8	4.5	138	77.5	10	5.6
III	349	92	26.4	58	16.6	34	9.7	70	20.1	34	9.7	17	4.9	289	82.8	74	21.2
<i>Intrauterine irradiator >100 mg Ra ek and/or vaginal irradiator >90 mg Ra ek at one or both treatment courses (L)</i>																	
I	230	45	19.5	30	13.0	15	6.5	47	18.3	15	6.5	3	1.3	164	71.3	139	60.4
II	202	61	30.2	70	34.6	41	20.3	46	22.8	29	14.4	15	7.4	106	52.5	93	46.0
III	299	152	50.8	92	30.8	60	20.1	119	39.8	48	16.1	26	8.7	171	57.2	149	50.2
B 1970-1972																	
<i>Intrauterine irradiator <100 mg Ra ek, vaginal irradiator <90 mg Ra ek at both treatment courses (L)</i>																	
I	106	24	22.6	18	17.0	6	5.7	22	20.8	6	5.7	7	6.6	86	81.3	7	6.6
II	115	50	43.5	30	26.1	20	17.4	35	30.4	15	13.0	17	14.8	86	74.8	1	0.9
<i>Intrauterine irradiator >100 mg Ra ek and/or vaginal irradiator >90 mg Ra ek at one or both treatment courses (L)</i>																	
I	27	12	44.4	10	37.0	2	7.4	10	37.0	2	7.4	7	25.9	18	66.7	0	0.0
II	25	21	84.0	10	40.0	11	44.0	15	60.0	7	28.0	3	12.0	13	52.0	0	0.0

Group I Prophylactic treatment * Group II Controls

radiation injury and residual or recurrent tumour growth. The prophylactic treatment with alkoxyglycerols in combination with radiotherapy apparently prevents the development of radiation damage and the growth of the tumour separately or combined. Non prophylactic administration of alkoxyglycerols does not seem to influence the tumour growth—but still protects against radiation damage.

It has been worth while to include the complex injuries in this study since the incidence of these injuries is characteristic for the different groups. A great reduction was seen in case of prophylactic administration with alkoxyglycerols (group I) while no reduction was observed when these compounds were given only during the radiation treatment (group II).

The complex injuries (C) are in all the studied situations reduced to about 1/3 when alkoxyglycerols are administered before, during and after radiation treatment. The difference between the incidences of complex injuries in group I (prophylactic group) and group III (control group) is statistically significant $p < 0.001$. Prophylactic administration is therefore of paramount importance for the reduction of the incidence of total injuries and especially for the reduction of complex injuries. The adminis-

tration of alkoxyglycerols merely during and after radiation therapy does not reduce the incidence of these injuries.

In this connection it is of interest to mention that the capacity for forming antibodies after vaccination can be influenced by the administration of alkoxyglycerols. This fact was observed in a study in which patients were vaccinated against typhus paratyphus one day before and one day after the implantation of radium. In the group given alkoxyglycerols certain antibodies were formed to a greater extent than in the group receiving merely radiation treatment (4). It is therefore probable that the general immunological response can be enhanced by treatment with alkoxyglycerols and thereby the defence mechanism of the body against malignant cells intensified.

The introduction of the concept of complex injuries facilitates a probable explanation why the incidence of radiation injuries is higher in the prophylactic group than in the non prophylactic group.

As mentioned before, no decrease of incidence of complex injuries is observed when alkoxyglycerols are administered only during radiation treatment. However, there is a remarkable effect on the radiation injuries which are reduced from 78% to 11%.

if all patients are taken into consideration the decrease of radiation injuries is even slightly higher than for the prophylactic group Tables III A and VII A. The suggested explanation would be that complex injuries (C) are representative of two components: one C_{R+T} is due to radiation damage and tumour growth in combination and the other is due to tumour growth alone. If for a proportion of the injuries $C_{R+T}+C_T$ the C_T components are reduced or eliminated, the residue of the complex injury would be interpreted as a radiation injury (R). It is thereby inferred that a transformation from a complex to a radiation injury can occur if alkoxyglycerols are given in prophylactic administration but not if they are given only during and after therapy.

The incidence of complex injuries has an additional facet of interest. It has namely been found that about 99% of the patients with complex injuries will be dead within five years after the initiation of radiotherapy. Thus a decrease of complex injuries would likely correspond to a considerable increase in the five year survival rate. Data are available that support this contention.

By a comparison of the two intracavitary treatment modalities L and H respectively it is found that the incidence of injuries is considerably higher following the employment of increased rather than conventional amounts of radium. This despite the fact that the majority of the patients in the H group (about 85% of them) have received a calculated dose on the bladder wall <5000 rad and on the rectal wall <4000 rad. This underlines the statement made above that the increased amount of intracavitary radium was used in advanced bulky tumours. It is therefore not surprising that the incidence of injuries is more dependent on the amount of radium (times application time) than on the calculated dose contribution to the bladder or rectal walls.

One of the most striking effects of prophylactic administration of alkoxyglycerols described in this investigation is that it produced a decrease with more than 60% of the incidence of total injuries (I) in the group treated with increased amount of radium in the intracavitary irradiator.

The data described in the study strongly suggest that alkoxyglycerols have two distinct effects in relation of tumour growth and protection against tissue damage following radiation. The study of the so called complex injuries and the comparison be-

tween prophylactic and non prophylactic administration of alkoxyglycerols have made it possible to separate these two effects.

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PREOPERATIVE IRRADIATION IN STAGE III CARCINOMA OF THE OVARY

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Abstract A series of 456 patients with malignant epithelial tumors of the ovary clinical stage III is presented. Of the 96 were initially inoperable and 49 had a clinical gross of inoperable disease at the first examination. In 3000 rads external highvoltage irradiation it was as possible to remove the primary tumour the uterus (the omentum in 36 (38%)) of the previously inoperable cases. Of the 49 patients who had only a clinical gross of inoperability only four were inoperable at reoperation performed three weeks after completion of 30 rads external radiotherapy. The five year survival for the whole series was 16%.

The survival of patients with ovarian cancer is dependent upon several factors: the stage of the disease, the histological type and grade of malignancy, the amount of residual disease after surgery. It is a common situation in referral institutions to see patients with ovarian cancer who have had a laparotomy with only a biopsy performed due to inoperable disease. It has been claimed that even in patients with inoperable tumours as much tumour as possible should be removed without risk of life threatening complications (10). The value of preoperative irradiation is still under debate. This paper reports a prospectively designed study to determine whether such inoperable patients would benefit from preoperative external radiotherapy followed by a second attempt at removal of tumour.

MATERIAL

Between 1968 and 1973 a total of 1427 patients with malignant ovarian tumours were treated at the Norwegian Radium Hospital, 456 of which had epithelial tumours. According to the FIGO Classification (7) the clinical status of these patients at admission to the hospital is given in Table 1. In one third of the cases it had been

possible at the primary surgical exploration to perform so-called complete pelvic surgery: total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy. In another third of the patients only parts of the primary tumour or metastases had been removed and in 21% of the cases the tumour was found completely inoperable and only a biopsy had been taken. A clinical diagnosis of inoperable disease without laparotomy had been made in 49 patients (10.7%). The clinical examination included chest X-ray, barium meal and enema, intravenous pyelogram, proctoscopy, peritoneal fluid cytology and/or fine needle aspiration biopsy from the tumour as described by Kjellgren et al. (6).

The patients were all caucasians. The youngest patient was 21 years and the oldest 77 years of age. Distribution by histological type is shown in Table 11. The age distribution of the patients within the different tumour groups varied little with a mean age between 53.3 and 60.3 years. No patients have been lost to follow up and all deaths have been recorded as cancer deaths. Cumulative survival curves have been made according to the life table method (2).

Treatment

The 145 patients who were considered inoperable at the admission to the hospital all received 3000 rads midplane dose supervoltage therapy extending to the T₁₂-L₁ region. The dose was delivered in 10 fractions through parallel

Table 1 Clinical material. Status at admission to the Norwegian Radium Hospital

Treatment group	Number	Per cent
Complete pelvic surgery	154	33.7
Partial tumour resection	157	34.5
Inoperable biopsy only	96	21.0
Clinical diagnosis of inoperable disease	49	10.7
Total	456	100.0

Total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy

Table II *Distribution by tumour type*

Tumour type	Number	Per cent
Serous carcinoma	256	56
Mucinous carcinoma	55	12
Endometroid carcinoma	77	17
Other or unclassified	68	15
Total	456	100

Table III *Outcome of second look operation after 3000 rads external irradiation for 96 patients initially found inoperable*

Type of surgery	Number	Per cent
Complete pelvic surgery	36	37.5
Partial tumor resection	26	27.1
Still inoperable	34	35.4
Total	96	100.0

opposed anterior and posterior portals over four weeks. The kidneys were shielded after 2000 rads. Three weeks after irradiation was completed a new attempt at radical surgery was made.

RESULTS

The results of postirradiation surgery for the 96 patients that were considered inoperable at the first laparotomy are summarized in Table III. In some cases only parts of the tumour masses could be removed and in many cases also the uterus had to be left behind. Others were still totally inoperable despite the irradiation. In 36 patients (38%) it was possible to remove the primary tumour, the uterus and the omentum (complete pelvic surgery). In another 26 patients (27%) it was possible to remove part of the primary tumour and metastases. In 34

cases (35%) irradiation therapy had not influenced the operability of the tumour.

The 49 patients that had only a clinical diagnosis of inoperable disease without laparotomy initially all gave the impression of advanced disease. We cannot know for certain whether operability was enhanced by the preoperative radiotherapy. It should be noted however that there were very few (4 patients) that were completely inoperable in this group after having received 3000 rads external irradiation. In Table IV this group is compared with 407 patients who had some type of primary surgery performed before admission to the Norwegian Radium Hospital. As can be seen only 8% of the cases diagnosed clinically as inoperable were found completely inoperable after preoperative radiotherapy as compared to 34% of those cases who had surgical exploration before referral to our institution. The highest survival rate was found amongst those patients who had so-called complete pelvic surgery at the first attempt. Considering that they all had widespread disease with metastases to the upper abdomen a five year survival of 24% is not too disappointing (Table V). The corresponding survival rate for those who were considered inoperable at first laparotomy and in whom complete pelvic surgery could be performed at the second look operation after 3000 rads external irradiation was 13%. A similar survival rate was found in the group of patients who had a clinical diagnosis of inoperability before irradiation at the second look operation (15%). It is hardly surprising that the worst prognosis was found in the group of patients who were found to have inoperable tumour both at the first and second operation (5%). Fig. 1 shows the survival curves for 14 patients who had complete pelvic surgery performed at the first operation and for that group of patients

Table IV *Results of first surgical exploration with or without preoperative irradiation*

Type of surgery	No preoperative radiotherapy		Clinical diagnosis of inoperability and preoperative irradiation 3000 rads	
	No	Per cent	No	Per cent
Complete pelvic surgery	154	37.8	24	49.0
Partial tumour resection	157	38.6	21	42.9
Inoperable	96	23.6	4	8.1
Total	407	100.0	49	100.0

Table V. Survival rates in relation to treatment and operability

Treatment group	Survival			
	No	1 year (%)	2 years (%)	5 years (%)
Complete pelvic surgery at first laparotomy				
Preoperative irradiation	154	51	31	4
Operable at first laparotomy. Complete pelvic surgery after 3000 rads	36	4	7	11
Operable at first and second laparotomy				
3000 rads between laparotomies	34	8	17	5
Clinical diagnosis of inoperability				
3000 rads before laparotomy	49	45	3	15

10 were found inoperable both at the first and at second look operation

DISCUSSION

Preoperative radiotherapy has been strongly advocated by some authors (8-9, 12) to enhance operability and to decrease the probability of dissemination of tumour at surgery. This study demonstrated that operability is enhanced by preoperative radiotherapy making possible complete pelvic surgery in 34% of the patients initially considered inoperable. Our protocol included an attitude of aggressive surgery and different surgeons were involved in the laparotomies. These facts of course will influence the results and we cannot know how much preoperative radiotherapy has really changed the

operability in these patients. Furthermore it is interesting to note that only 8% of the patients that had a clinical diagnosis of inoperability upon admission were completely inoperable after radiotherapy. This figure should be compared to the 24% in operability in the 407 patients who had primary surgery elsewhere and who had no preoperative treatment.

This aggressive attitude however has its price. Among the 145 patients in the study group there were 13 postoperative deaths giving a postoperative mortality of 9% (defined as death within 30 days of surgery). Treatment of advanced disease of this type carries a high risk regardless of treatment modality. This is evident from the steep fall of survival curves in stage III ovarian cancer within the first few months following diagnosis. Removing the bulk of tumour is a simple question in patients with small mobile tumours. As other authors have recommended (3, 11, 13) we attempted to remove the uterus, both ovaries, the omentum and metastases that could easily be taken out. Median survival time is believed to be inversely proportional to the bulk of remaining tumour (4). Munnell (10) compared survival rates from 1952-1961 with earlier series and concluded that the improved survival rate was probably due to more aggressive surgery and radiotherapy. In the present study many patients had widespread disease throughout the upper abdomen. One may question if aggressive surgery is appropriate also for these cases. Some feel that so few of these patients can be salvaged that aggressive surgery is not the preferred means of therapy (1, 9). Kottmeier (8) makes one exception to the principle of conservative surgery in widespread disease. When the tumour is of the mucinous type the response to radiotherapy will be

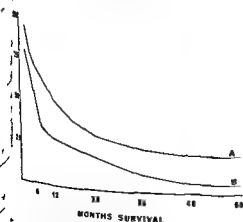


Fig. 1. (A) Survival curve for 154 patients initially operable with no preoperative treatment. (B) Survival curve for 34 patients initially inoperable at first laparotomy. 3000 rads external radiotherapy to large abdominal field before second laparotomy. All were still inoperable.

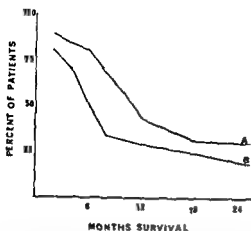


Fig 2 Survival curves for initially inoperable patients (A) 36 patients that had complete pelvic surgery at second laparotomy after receiving 3000 rads external radiotherapy (B) 34 patients inoperable at first and second laparotomy 3000 rads external radiotherapy between laparotomies

less and one should in such cases depend more upon surgery. In this study the majority of the mucinous tumours (78%) were primarily operable and only eight patients in the study group had mucinous tumours. None of these patients survived more than 36 months.

If five year survival is used as a criterion to evaluate the benefits of aggressive surgery following preoperative radiotherapy we can only show a small increase in salvage rates. In those patients who had successful surgery at the second attempt however there was a shift towards prolonged survival for the first two years compared to those that were still inoperable (Fig. 2). One can only hope that this brief postponement of death was combined with some degree of palliation of symptoms.

It may be concluded that operability is undoubtedly influenced by preoperative radiotherapy but increased survival attributable to aggressive surgery was not as great as anticipated.

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VARIATIONS IN PLASMA STEROID AND PROSTAGLANDIN CONCENTRATIONS DURING HUMAN PREGNANCY

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Abstract Radioimmunoassay of sex steroids and prostaglandins was performed on plasma obtained from 10 uncomplicated primigravid subjects at a stated time of the day in varying stages of gestation. Prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), oestrone and oestrol concentrations reached a peak a few weeks before term and then declined. Progesterone, 17 hydroxyprogesterone and oestradiol increased during pregnancy particularly after 34 weeks. In two normal patients repeated blood samples were taken throughout the day at weekly intervals in late pregnancy for sex steroid assays to evaluate the variation of concentrations with the time of sampling and the significance of changes in mean concentrations of different steroids with approaching parturition. Marked variation was found in the levels of different steroids during the day. Plasma oestrol values were lowest at 8.00 a.m. in the patient sampled frequently. A marked decrease in oestrol, oestrone, oestradiol and 17 hydroxyprogesterone was found in one patient prior to the onset of spontaneous labour. In another subject there was no decrease in concentrations of oestrogens or progestogens prior to induction of labour at term.

Although sensitive radioimmunoassay techniques have been in use for several years, there is still no general agreement as to what precise changes occur in the endocrinological status in late pregnancy. In

some animal species blood progesterone levels fall preceding the onset of labour (8) but only a few studies have confirmed this in man (3, 10). Oestrogen levels rise towards term and oestradiol promotes prostaglandin synthesis (2). Prostaglandins stimulate the myometrium and enhance and potentiate endogenously released oxytocin (5). However the exact interrelationship and interdependence between the various protein and steroid hormones and prostaglandins during pregnancy is imprecise.

This study was undertaken to establish the levels of sex steroids and prostaglandins in uncomplicated pregnancies throughout gestation and particularly to assess whether the results are influenced by the time of sampling. Therefore, in late pregnancy serial samples were taken from specific individual subjects to help assess this possibility.

MATERIALS AND METHODS

Healthy primigravid subjects with an uncomplicated pregnancy acted in a volunteer capacity. Each patient had

Table I Variations in sex steroid concentrations (with time of sampling)

Plasma hormone (ng/ml)	Time of sampling				
	8 p.m.	12 p.m.	8 a.m.	12 noon	4 p.m.
Progesterone	33.2	83.4	52.0	76.4	61.3
17 hydroxyprogesterone	74.8	22.9	20.0	26.4	24.4
Oestrone	11.4	10.7	13.7	13.0	21.3
Oestradiol	13.7	17.9	14.7	18.8	23.1
Oestrol	7.1	12.3	3.7	10.1	8.1

Lowest value

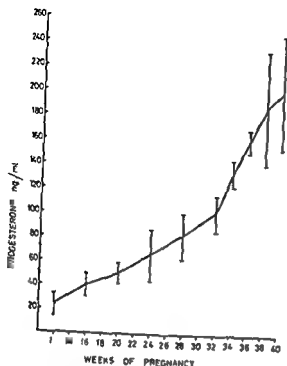
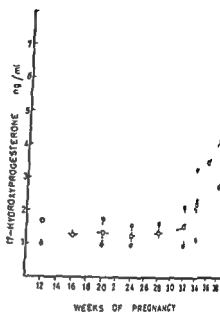


Fig 1 Changes in plasma progesterone and 17 hydroxy progesterone during pregnancy



impeccable menstrual data to indicate that her gestation was correct. In each instance there was no discrepancy greater than one week between the clinical evaluation of gestation and the menstrual age of the pregnancy. Ten cross sectional samples were taken in routine antenatal clinics between 9.00 a.m. and 10.00 a.m. once each month from 12 weeks to 28 weeks. After 28 weeks samples were taken at two weekly intervals until 36 weeks and weekly thereafter.

In addition two normal primigravid patients acted as volunteers to assess the possible variation in steroid levels with the time of sampling. They were admitted at weekly intervals from 35 weeks onwards and an intravenous can-

nula was inserted into a forearm vein. Samples of blood were collected four hourly from 8.00 p.m. on one day 4.00 p.m. on the following day omitting the 4.00 a.m. sample. The patients were then allowed home.

10 ml of heparinised blood was taken from the antecubital vein by venepuncture or from an intravenous cannula in those patients sampled throughout the 24 hours. The plasma was separated following centrifugation and stored at -70°C until assayed. Radioimmunoassays for oestrogens, progesterone, 17 hydroxyprogesterone and $\text{PGF}_{2\alpha}$ were as reported previously (11, 17, 13, 14). $\text{PGF}_{2\alpha}$ concentrations were evaluated using a technique similar to that of $\text{PGF}_{2\alpha}$ (15).

Table II Variation in progesterone with time of sampling at weekly intervals in late pregnancy

Gestation (wk)	Time of sampling				
	8 p.m.	12 p.m.	8 a.m.	12 noon	4 p.m.
34	48	56	53	46	51
35	93	71	51	62	57
36	93	65	44	54	62
37	33	83	52	76	61
38	159	150	118	122	109
39	116	141	110	130	148
40	164	137	114	161	128

Lowest value

Table III Variations in oestriol with time of sampling at weekly intervals in late pregnancy

Gestation (wk)	Time of sampling				
	8 p.m.	12 p.m.	8 a.m.	12 noon	4 p.m.
34	2.3	3.3	0.5	1.7	3.7
35	4.6	6.3	2.7	3.7	4.8
36	16.3	5.2	4.3	4.7	5.6
37	7.1	17.3	3.7	10.1	8.1
38	12.2	7.3	6	8.0	6.7
39	7.7	14.5	6.6	5.3	13.9
40	7.2	12.3	6.9*	17.5	14.0

* Lowest value

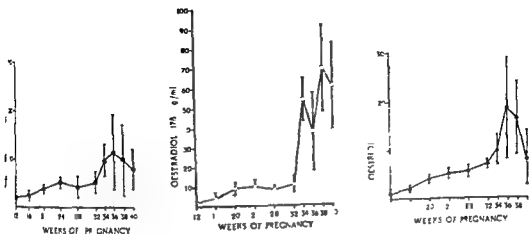


Fig 2 Changes in plasma oestrogens during pregnancy

RESULTS

There was a continuing rise in progesterone levels throughout pregnancy up to term and an increase in 17-hydroxyprogesterone particularly marked after 32 weeks (Fig 1). In this study circulating oestrone and oestradiol levels increased to a peak between 34 and 36 weeks and then declined gradually to term (Fig 2). Oestradiol values showed a particular elevation after 32 weeks. Prostaglandin E_2 and $F_{2\alpha}$ concentrations similarly reached a peak several weeks before term and then gradually declined (Fig 3).

The variation in steroid concentrations with the time of sampling in a specific patient at 37 weeks is

seen in Table I. Progesterone levels showed a marked variation throughout the day from 33.2 to 83.4 ng/ml whilst 17-hydroxyprogesterone levels exhibited less fluctuation. The variation in oestrol concentrations was twice as great as for oestradiol and oestrone.

Variations in the change in concentrations of progesterone from the time of sampling at different weeks of gestation in late pregnancy are seen in the same subject in Table II. Four out of seven records were lowest at 8.00 a.m. and in two of the other three the 8.00 a.m. value was the next lowest.

Variations in the oestrol values in the same sub-

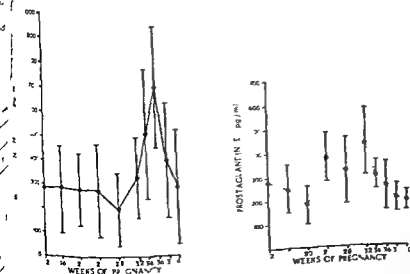


Fig 3 Changes in plasma prostaglandin E_2 and prostaglandin $F_{2\alpha}$ during pregnancy

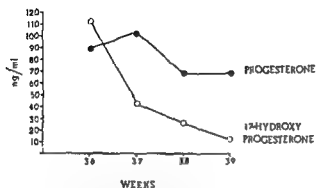
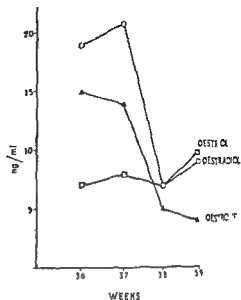


Fig 4 Changes in mean plasma steroid concentrations of serial daily samples preceding spontaneous labour

ject expressed in the same manner are seen in Table III. The majority of low values were also found at 8.00 a.m.

The mean concentrations of the five daily samples taken from two subjects serially sampled at weekly intervals in late pregnancy are seen in Figs 4 and 5 respectively. The patient shown in Fig 4 went into spontaneous labour five days after the last sampling. 17-hydroxyprogesterone, oestrone and oestradiol values declined prior to the onset of spontaneous labour, whereas progesterone values



remained relatively static. In the other subject (Fig 5) labour was induced for social reasons shortly after term but until this time oestrone, oestradiol and progesterone continued to rise, oestrone and 17-hydroxyprogesterone varied very little.

DISCUSSION

Plasma progesterone values increased up to pregnancy to term and no dramatic decline in concentrations was observed in the two patients se-

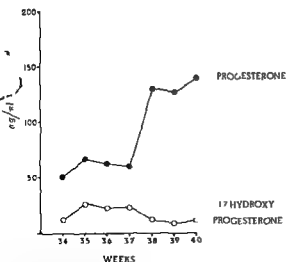
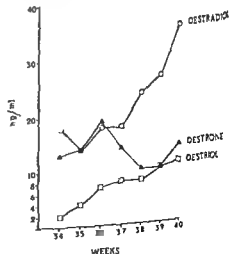


Fig 5 Changes in mean plasma steroid concentrations of serial daily samples preceding induction of labour at term



ly sampled in the last month of pregnancy. These results are contrary to those reported by Csapo (1) and Turnbull (10) but isolated rather than repeated samples were taken in these studies. The variation found in the samples taken in individual subjects at different times of the day confirming previously reported by one of us (1). Collection of samples in the early morning when the variations appeared least indicates that this may be an optimal time for sampling if the results are to be meaningful. 17 hydroxyprogesterone concentrations varied less throughout the day in individual subjects studied but as with progesterone a continuous upward trend was seen as gestation progressed particularly after 37 weeks as has been reported by (9).

(9) The significance of this steroid in pregnancy and during parturition has yet to be evaluated since its production has been largely considered up to the present to be associated with the function of the corpus luteum. However the marked decline in mean concentrations of 17 hydroxyprogesterone, oestrone and oestradiol in the event going into spontaneous labour may indicate alterations in the metabolic pathways of these steroids in the foeto-placental unit with incipient labour.

The finding that there is no significant diurnal variation in plasma oestrol values in human pregnancy is in accordance with another study (7). Oestrogen production by the placenta has been suggested to trigger the synthesis of prostaglandins (2) and PGE_2 and $\text{PGF}_{2\alpha}$ concentrations were found to show the pattern of increase in oestradiol levels after 37 weeks. However circulating prostaglandin levels did not remain elevated after 36 weeks but declined possibly due to an effect of high circulating progesterone concentrations or perhaps some yet isolated lysosome stabilising factor (6). The exact relationship between natural prostaglandins, the more stable metabolite 13, 14 dihydro 15 keto $\text{PGF}_{2\alpha}$ and sex steroids prior to the onset of spontaneous labour in human pregnancy awaits elucidation. The variability of results with the time of sampling indicates that there are limitations in interpreting peripheral blood steroid concentrations as either a predictive test of incipient fetal demise or for assessing the likelihood of the onset of parturition. It is therefore not surprising that it is difficult to draw significant conclusions from apparent inter-relationships of different hormones at a site distant from the uterus.

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rekommenderar
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patienter



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ställas om p.g.a.
biverkningar med
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Neovletta

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THE CHANGE OF ALKALINE PHOSPHATASE BINDING CONDITIONS WITH TROPHOBLAST MEMBRANE AT DIFFERENT STAGES OF HUMAN PLACENTA

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Alkaline phosphatase was brought into solution from microsomal fractions of placentas of varying gestational age by using gradually increasing concentrations of papain. When the activity of the soluble alkaline phosphatase (S) was compared with that of the residue (R) the S/R ratio rose as pregnancy progressed. The electrophoretic pattern showed that in serum from pregnant women the papain soluble alkaline phosphatase corresponded to the heat stable one. These results indicate that the cytoplasmic membrane of the trophoblast changes with the growth of the placenta so that this enzyme is easily dissolved by papain. It is probable that alkaline phosphatase molecules easily enter the maternal blood stream in late pregnancy.

Human placental alkaline phosphatase activity in the serum of pregnant women increases as pregnancy progresses and this phenomenon has been widely used clinically as a test of placental growth and function as this enzyme is distinguished from others by its heat stability (1, 4, 5, 6, 7, 9). As the specific activity of the placental alkaline phosphatase increases with placental growth the activity of this enzyme may also increase in maternal serum (5) though this relationship has not been proved. The alkaline phosphatase releasing mechanism may involve changes in the placental trophoblast membrane as it grows since alkaline phosphatase binds to the membrane of the trophoblast (5). In the present study human placental microsomes of varying gestational age were isolated and treated with proteinase papain. The ratio of the activity of soluble alkaline phosphatase to that of the residue bound to microsomes was calculated. Furthermore soluble alkaline phosphatase was compared with placental

alkaline phosphatase in the serum of pregnant women by polyacrylamide gel electrophoresis. The releasing mechanism of this enzyme from the placenta was discussed.

MATERIALS AND METHODS

Human placenta Placentas were obtained from cases of normal delivery, spontaneous abortion and induced abortion. None of the cases were complicated by, for example, toxemia, diabetes mellitus, etc.

Microsome preparation All placentas were stocked immediately after delivery in a deep freezer at -70°C and thawed at room temperature for use. After thawing they were minced with scissors and washed in 0.9% NaCl solution and blood, connective tissue and vessels were removed as far as possible. The chopped tissue was homogenized with Teflon homogenizer by five up-and-down strokes in 10 mM Tris-Cl pH 7.2 at 0°C - 4°C . The homogenates were centrifuged at $3000 \times g$ for 15 min at 4°C and the supernatants were centrifuged again at $105000 \times g$ (R_{100}) for 1 hour at 4°C . The microsomal pellets were suspended in 10 mM Tris-Cl at pH 7.2.

Papain treatment to bring alkaline phosphatase into solution Ten ml of papain solution (Merck 3.5 mg Anson E/mg in 10 mM Tris-Cl pH 7.2) was prepared in gradual concentrations. These were added to 0.1 ml of the microsomal fractions (0.63 mg of protein) of varying ages incubated at 36°C for 30 min and centrifuged at $105000 \times g$ (R_{100}) at 4°C . After centrifugation the pellets were suspended in 3.0 ml of 10 mM Tris-Cl pH 7.2. Aliquots 0.1 ml of each of the supernatant and pellet suspensions were used for the measurement of the alkaline phosphatase activity by King & Kinds method at pH 10.1 (2).

Determination of protein concentration Protein determination of microsomes was performed by Lowry's method using bovine serum albumin (type V) as a standard (3).

Polyacrylamide gel electrophoresis 0.1 ml aliquots of

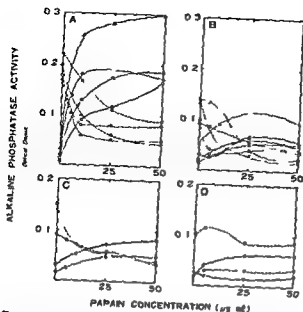


Fig 1 The activities of the soluble alkaline phosphatase and those of the non soluble residue. Measurement of alkaline phosphatase activity was performed by kind & Kings method using wavelength 580 nm. The same plot marks represent the same placenta. Week of gestation (A) 36-40 weeks (B) 25-28 weeks (\times 32 weeks) (C) 21-24 weeks (D) 13-16 weeks — Soluble fraction — non soluble residue

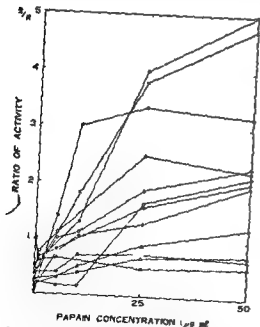


Fig 2 Ratio of activities of soluble alkaline phosphatase (S) and that of the non soluble residue (R). Aliquots 0.1 ml from 10 ml of soluble fractions and 3.0 ml of non soluble residue suspensions were used for the measurement of enzyme activity. The true S/R ratio is calculated by multiplying the values in Fig 2 by 10/3. \times 36-40 weeks \circ 25-28 weeks Δ 21-24 weeks \triangle 13-16 weeks



Fig 3 Electrophoretic zymogram patterns of alkaline phosphatase (A) Papain soluble fraction from placental microsome (B) Heat treated serum of a late pregnant woman

papain solubilized solution from the mature placental microsome and the serum of pregnant women were fixed onto 7.5% polyacrylamide gel buffered by 10 mM Tris, pH 8.0 and treated electrophoretically at 140 volts for 15 min. (The serum was preheated at 56°C for 15 min to inhibit the alkaline phosphatase from other sources). Alkaline phosphatase was stained in the solution containing 10 mM Tris Cl, pH 8.5, 0.1 mg/ml of alpha-naphthylphosphate disodium salt as substrate, 1% dimethylformamide and 0.3 mg/ml of Fast blue BB salt (Sigma).

RESULTS

Rate at which alkaline phosphatase from placental microsomes of varying age was brought into solution by papain. Fig 1 reveals the activity of papain soluble alkaline phosphatase and the non-soluble enzyme from placental microsomes of varying gestational age. As the placenta grows, the activity of soluble alkaline phosphatase rises and reaches a constant value at a low concentration of papain. In order to simplify the interpretation

g. 1 the activity of papain soluble alkaline phosphatase (S) and that of the non soluble residue (R) were calculated (Fig. 2). Not only does the activity of alkaline phosphatase increase with the S/R ratio also increases with placental growth.

Electrophoretic comparison of papain soluble alkaline phosphatase with that in serum of pregnant women. To compare papain soluble alkaline phosphatases with that of placental enzyme in the serum of pregnant women both were loaded onto 10% polyacrylamide gel for electrophoresis (the serum was pre heated at 56°C for 15 min). The alkaline phosphatase was stained as described in Materials and Methods. No difference was found in the electrophoretic distance between papain soluble alkaline phosphatase and the placental enzyme in the serum (Fig. 3). Therefore papain soluble alkaline phosphatase from the placenta resembles the enzyme in the serum of pregnant women.

DISCUSSION

It is well known that the activity of placental alkaline phosphatase rises in the serum of pregnant women (1, 4, 5, 6, 7, 9). This enzyme is present in the microvilli of syncytiotrophoblast and increases as gestation progresses (5). The microvilli lie in close contact with the maternal blood and consequently alkaline phosphatase molecules can enter the maternal blood stream via the microvilli (10). Another mechanism may be considered. The present results show that the volume of papain soluble alkaline phosphatase from placental microvilli increases as gestation progresses and the soluble enzyme resembles that from the placenta in the serum of pregnant women in its electrophoretic mobility. The proteins bound superficially to the cell membrane are reported to be brought in solution by papain (8). Therefore these results indicate that the trophoblastic membrane changes as the placenta grows. The binding conditions for alkaline phosphatase molecules to the cell surface change so


that the enzyme is more easily brought into solution by papain. Probably more alkaline phosphatase molecules on the cell surfaces enter the maternal blood stream in late pregnancy.

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 **FERROSAN**

HYPERTHYROIDISM DURING PREGNANCY TREATED WITH PROPYLTHIOURACIL

The Significance of Maternal and Foetal Parameters

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Hyperthyroidism during pregnancy may be dangerous to the infant. The major risks are prematurity and neonatal thyrotoxicosis. The latter may be due to placental transfer of thyroid stimulating immunoglobulins from mother to fetus. Of two siblings of a previously thyrotoxic mother the first had marked symptoms of neonatal thyrotoxicosis after a pregnancy where no antithyroid treatment was given. The second child had only minimal thyrotoxic symptoms but almost as high levels of thyroid hormones as the first. During the second pregnancy propylthiouracil was given to the mother from 26 weeks gestation because of increased fetal movements and fetal tachycardia. Fetal movements and fetal heart rate were considered to be most valuable indicators of thyroid function in the fetus. Intense control is necessary from the second trimester.

Hyperthyroidism during pregnancy imposes little risk to the mother but it may be dangerous to the fetus and newborn. The incidence of toxæmia has been reported as higher (11). However the major risks are the marked increased incidence of prematurity with its resultant complications and mortality and the risk of neonatal thyrotoxicosis with a mortality of 16 percent in the described 75 cases (12). The physical signs of thyrotoxic disease in the newborn may be absent at birth but will appear within the first few days or weeks of life. The initial symptoms are restlessness and diarrhoea. Later on the infant may develop tachycardia, tachypnoea, enlargement of the thyroid and exophthalmos. It may fail to gain weight in spite of adequate caloric intake. The disease is often transient and benign but may be lethal with progressing cardiac failure.

Many observations suggest that some humoral factor transferred placentally from mother to fetus is responsible for the symptoms in the infant. Maternal long acting thyroid stimulator (LATS) has until recently been accepted as the pathogenic factor (4-12). Antithyroid medication during pregnancy may reduce morbidity and mortality substantially (14). However overtreatment may be dangerous to the fetus with resultant hyperthyroidism with mental retardation and goitre. Most careful physical and laboratory control during pregnancy is therefore essential.

METHODS

Total serum thyroxine concentration (T_4) was assayed using the Thyopac* 5 kit from The Radiochemical Centre, Amersham, England. Normal ranges were 65-145 nmol/l in adults and 65-175 nmol/l in pregnant women.

Free thyroxine (FT_4) was assayed as normalized thyroxine ratio by the same Thyopac* 5 kit. Normal range in adults and pregnant women was 0.88-1.11 rel. U.

Serum triiodothyronine (T_3) was assayed using The Radiochemical Centre's T_3 kit. Normal ranges were 1.3-3.5 nmol/l in adults and 1.5-5.0 nmol/l in pregnant women.

Long acting thyroid stimulator (LATS) was assayed using white guinea pigs (7). In this technique LATS values below 1.25 rel. U. were normal, 1.25-1.50 was suspected, elevated and more than 1.50 was considered significant pathology.

Fetal heart rate was detected with the Hewlett Packard Cardiotocograph 8071 A Recorder 8025 B.

Fetal movements were judged by external physical examination.

Table I Second pregnancy Maternal and fetal laboratory and physical parameters before and during treatment with propylthiouracil

B=birth rel U=relative unit HPL=human placenta lactogen

	Gestational age (weeks)										C Bl
	18	22	24	26	28	30	32	34	36	37 (B)	
T ₄ (nmol/l)	264	230	223	269	138	120	130	134	115	169	21
FT ₄ (rel U)	1.22	1.18	1.20	1.25	0.98	0.96	0.95	1.0	0.94	1.0	1
T ₃ (nmol/l)	>7	>7	>7	>9	4.5	—	5.9	5.1	—	—	1
LATS (rel U)	0.87	0.87	1.40	1.56	0.83	0.76	—	0.76	1.38	1.70	0
U oestrol (μmol/24 h)	—	—	49	73	50	81	95	101	115	—	—
S HPL (g/l)	—	—	5.0	3.3	5.3	4.8	5.7	5.3	5.4	—	—
Fetal heart rate per min	140	150	155	165	145	140	135	140	140	135	—
Fetal movements	0	+	++	+++	+	+	+	+	+	+	—
Propylthiouracil (mg/24 h)	0	0	0	300	150	75	62.5	62.5	67.5	67.5	—

CASE HISTORY

The mother 27 year old gravida 2 para 2 aborta 0. In 1966 she had undergone partial thyroidectomy for exophthalmic goitre with marked thyrotoxic symptoms. She had since been well with no need of antithyroid treatment. The exophthalmos persisted and a minimal thyroid enlargement developed again in the years following operation.

Non pregnant laboratory data December 1974 T₄ 153 FT₄ 1.13 November 1976 T₄ 143 FT₄ 1.10

First pregnancy Girl delivered February 1974 at 34 weeks gestation by Caesarean section indicated by prolapsed umbilical cord. During pregnancy no antithyroid treatment was given. The mother developed mild pre-eclampsia in the week preceding delivery. Birth weight was 2050 g and Apgar score was 10 at five minutes. Physical examination of the infant revealed staring eyes but no thyroid enlargement. During the first week she became hyperkinetic with loose stools. At 3 weeks age marked exophthalmos and moderate thyroid enlargement had developed. Heart rate was 180–200/min. She was treated in an incubator with 30% oxygen and phenobarbital. The thyrotoxic symptoms gradually subsided during the following weeks. The child was discharged in good condition 50 days old. Some exophthalmos persisted but she remained euthyroid.

Laboratory data at age 2 weeks T₄ 289 FT₄ 1.35 TSH <0.4 mU/l (normal). LATS could not be detected qualitatively by the method then available but was found qualitatively.

Laboratory data at age 10 weeks T₄ 139 FT₄ 1.07

Second pregnancy Boy born spontaneously August 1976 at 37 weeks gestation. During pregnancy the mother was under strict control in open ward from 11 weeks gestation. At 26 weeks gestation the fetus became hyperkinetic with tachycardia recorded by cardiotocograph. Treatment with propylthiouracil was started on physical indication (Table I). The further control and medication was based on maternal laboratory data, fetal movements and fetal heart rate. The mother did not become toxæmic and the circumference of her neck remained the same during the medication. The infant was

asphyxiated with Apgar score 5 at one minute and ten minutes. Birth weight 2500 g. The physical examination was normal. During the first weeks he developed slight lid retraction. No other thyrotoxic symptoms noticed. No specific treatment was required and the was discharged in good condition when 10 days old remained well.

Cord blood samples including LATS were all normal cord blood (Table I).

Laboratory data at age 7 weeks T₄ 277 FT₄ 1.36 T₃ 0.90

Laboratory data at age 10 weeks T₄ 83 FT₄ 0.83

DISCUSSION

The thyroid gland is developed in the first trimester from the thyroglossal duct which appears down growth from the floor of the mouth. In serum T₄ and FT₄ concentrations during pregnancies are low between 11 and 18 weeks gestation and increase progressively between 18 and 34 weeks gestation suggesting rapid maturation of fetal hypothalamic pituitary unit (5).

Maternal T₄ is increased throughout pregnancy due to an increase in thyroxine binding globulin (13). FT₄ remains normal for adults and is then the most reliable. Maternal thyroid stimulating hormone (TSH) increases during the first and second trimesters but returns to normal levels at 1 trimester. Radioimmunoassay of TSH may cross react with human chorionic gonadotropin and is therefore of limited value.

Cord blood samples from normal samples are equal or raised T₄, FT₄ and TSH levels compared with maternal blood (6). T₄ and FT₄ are temporarily elevated during the first few weeks of life with

response to any thyrotoxic symptoms in the normal newborn (1-16). In neonatal thyrotoxicosis the increase in the serum levels is higher and of longer duration than the physiologic

Clinical observations seem to indicate that neonatal thyrotoxicosis is due to some humoral factor transferred placentally from mother to fetus. Neonatal thyrotoxicosis is closely related to current or previous maternal thyrotoxic disease especially when associated with exophthalmos or pretibial edema in the mother. T_4 , T_3 and TSH cross the placenta poorly. LATS crosses well like other immunoglobulins. G LATS has been detected by bioassay in most thyrotoxic infants but not in all (3). Another immunoglobulin of maternal origin, human thyroid stimulating immunoglobulin (HTSI) - formerly known as long acting thyroid stimulator or 'LATS P' - has recently been detected by radioimmunoassay in thyrotoxic infants where no LATS was found (2, 15). HTSI is much more correlated to adult thyrotoxicosis than LATS (17). These observations are not necessarily contradictory. LATS may be an immunologic marker whereas HTSI is the pathogenic factor and vice versa. The two thyroid stimulating immunoglobulins now known may even both be immunologic markers of unknown events. The exact pathogenesis remains obscure.

In the second pregnancy the fetus became hyperthyroid and developed tachycardia (Table I). The symptoms were regarded as signs of fetal thyrotoxicosis. Antithyroid treatment with propylthiouracil which crosses the placenta well was started with immediate clinical and laboratory effect. The fetal thyrotoxic episode retrospectively has shown to be well correlated to a significant increase in maternal LATS level. LATS bioassay at 4-6 weeks and was therefore of little practical value. Immediately before birth LATS was again elevated but maternal T_4 and FT_4 remained normal. Cord blood LATS was normal and the newborn was not treated with no signs of overtreatment. He developed no signs of neonatal thyrotoxicosis except minimal lid retraction but nevertheless his T_4 and FT_4 values were temporarily elevated and almost analogous to those of his elder sister who was premature and clearly thyrotoxic born after a pregnancy where no antithyroid medication was given. The marked difference in clinical symptoms may be explained partly due to the peripheral effect of propylthiouracil which has been shown to block

the extrathyroidal conversion of T_4 to T_3 , the latter being the most biologically active hormone (8).

We suggest that fetal movements and fetal heart rate are most valuable indicators of thyroid function in the fetus. They were found to be reliable and practical parameters in the control of the hyperthyroid pregnancy and the eventual antithyroid medication. The control including serial detections of FT_4 should be strict from the beginning of the second trimester. Medical treatment with propylthiouracil until birth without concomitant thyroxine substitution is most logical and the treatment of choice today (7-14). Serial detections of LATS and HTSI should be done if available to support the neonatal paediatric care but at least LATS is of little practical value to the obstetrician.

The paediatric aspects of our cases are described in detail elsewhere (17).

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THE EFFECT OF DEXAMETHASONE THERAPY IN PROLONGED PREGNANCY

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Abstract Fifty six patients chosen by random sampling from a total group of 170 post term women received dexamethasone (Decadron® MSD) 2 mg 3 times a day for 4 wk, the other 64 patients acting as controls.

The evolution of uterine activity was evaluated using the score (PS) and a modified low dosage oxytocin sensitivity test (OST) before (T_1) and after (T_2) the treatment.

During the interval from the second to the 6th day inclusive after T_1 35 women of the dexamethasone group and 13 of the control group had a spontaneous onset of labour (SO) ($P < 0.01$).

Five patients in the dexamethasone group with primary rupture of membranes started labour spontaneously within 12 hours after membrane rupture. 7 patients in the control group with primary rupture of membranes received oxytocin as labour did not start within 24 hours.

Excluding patients artificially induced the mean interval from T_1 to SO was 6.8 days in the control group and 5.4 days in the dexamethasone group ($P < 0.001$).

In both groups PS and the sum of Montevideo units (MU) during OST increased from T_1 to T_2 , the increase was significantly greater in the dexamethasone group than in the control group. We found no correlation between the results of OST and the T_1 -SO interval. Dexamethasone used in this study may promote labour in prolonged human pregnancy. Due to its low potency it is not a substitute for oxytocin in the induction of labour.

The lowered placento-fetal quotient in the dexamethasone group warrants further study of the effects of steroid hormone on placental function.

In some animals corticosteroids have the ability to initiate labour (8). In post term women dexamethasone has been shown to promote labour (15) when injected into the amniotic fluid. By routine use of corticosteroid hormone induction of labour

(IL) the side effects of oxytocin and/or amniotomy (4, 10, 11, 20) could be avoided. We report the results of oral administration of dexamethasone to a group of post term women.

Patients

Only patients with singleton pregnancies, cephalic presentation and without maternal illness or complications of pregnancy were included. One patient was excluded because of twin pregnancy, another because of hypertonic uterine activity (Fig. 1).

Patients who had obviously not reached term were not included. Others were excluded as PS initially was too high to permit any appreciable increase. Thus a group of 120 post term patients with as much clinical uniformity as possible was selected (Table I). The controls had passed term by 3 days more than the dexamethasone group. This fact and the predominance of boys among nulliparous controls may explain the difference between the groups in the babies' weight (Table I).

The pregnancies were monitored by frequent amniocentesis, assay of estriol excretion and heat stable alkaline phosphatase or placental cystine aminopeptidase in maternal plasma. Ultrasound cephalometry was found useful especially to exclude preterm patients.

Assessment of PS at the second test (T_2) was the only factor which could be influenced by subjective bias. As corticosteroids are known to depress estriol excretion (2, 18, 21) we did not use a blind study model.

The babies' weights were recorded to the nearest 5 g, placental weight to the nearest 10 g, after late clamping of the umbilical cord.

Experimental Procedure

The nature of the experiment was explained to each patient. She was placed in a comfortable and stable supine position with at least 15° left tilt. The pressure receptor of a Malmstrom parturiometer (14) was applied to the maternal abdomen while confirming proper positioning and tightening. The air pressure of the parturiometer was recorded by an Elema Schönander transducer EMT35. The signal was written on a Siemens Kompensograph III; the

Abbreviations IL: artificial induction of labour; MU: Montevideo units; OST: Oxytocin sensitivity test; PS: pelvic score; T_1 : First test; T_2 : Second test; \square : Spontaneous onset of labour.

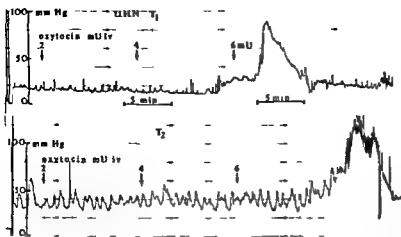


Fig. 1 Gravid 1 9 days past term 6. During both tests tetanic uterine traction occurred after 6 mU of oxytocin. Administration of oxytocin was stopped and tetanic contractions did not recur. Recording continued for one hour during both tests. Normal vaginal delivery 5 days after T_1 .

excursion of the pen having been calibrated against a column of mercury.

The oxytocin sensitivity test (OST) of Smyth (10 mU oxytocin intravenously every minute until uterine contraction occurs) seems to reflect myometrial function accurately in terms of its response to the induction of labour (22). As we have witnessed several cases of tetanic uterine contraction while using the oxytocin dosage recommended by Smyth, we lowered the dose, maintaining the simplicity of the original method.

After a control period the patient was given single injections intravenously of 2, 4, 6, 8, 10 and 12 mU oxytocin (Syntocinon® Sandoz) at 10 min intervals. The activity of the myometrium was calculated in Montevideo units (MU) as the sum of pressure increase in the contractions during each 10 min period, disregarding deviation of the pressure curve of less than 5 mmHg. The Malmström parturimeter has been shown to give a fairly accurate record of the amniotic pressure during contractions (5). This external method of recording was chosen in order to avoid myometrial stimulation by using an intra- or extra-amniotic catheter.

Using the method of Burnett (3) pelvic score (PS) was assessed after the OST. The cervical position, effacement, dilatation and consistency and the station of the fetal head were given scores 0, 1 or 2; the sum of PS ranging from 0 to 10.

After the completion of this first test (T_1) the patients were allocated at random to a treatment group receiving dexamethasone (Decadron® MSD) 2 mg three times each day for 4 days, total dose 24 mg, the first test being the first treatment day. Patients allocated to the control group received no treatment.

On the fifth day the OST and assessment of PS were repeated (T_2). We confirmed that dexamethasone had been taken as prescribed. All tests were performed in the morning to avoid the influence of endogenous corticosteroid diurnal variation.

The patients in the two groups were compared statistically with the χ^2 test. Differences between the means were evaluated using t from the formula:

$$t = \frac{X_1 - X_2}{SE}$$

where

$$SE_{diff} = \sqrt{\frac{\sum(x_1 - \bar{x}_1)^2 + \sum(x_2 - \bar{x}_2)^2}{(n_1 - 1) + (n_2 - 1)}}$$

Correlation coefficients were calculated with a calculator (CompuCorp Statistician). $P < 0.05$ was considered statistically significant.

Table 1 Clinical details of patients and babies

	No. of patients	Mean age (years)	Days post calc term at T_1	No. of patients with IL	Babies		
					Boys/girls	Mean weight (g)	Apgar score 1 min
Control group	64						
Nulliparae	40	24.5	13.3	11	27/18	3809	8.5
Multiparae	24	26.8	11.1	17	14/10	3619	8.3
Dexamethasone group	56					3601	
Nulliparae	29	24.5	10.8	5	1		
Multiparae	27	27.6	8.0	7	1		

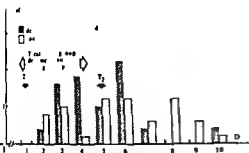


Fig. 2 Number of patients with spontaneous start of labour (SO) each day on the 10 days following T_1 . On days 1-6 inclusive 35 dexamethasone treated and 15 control patients started labour spontaneously. On days 7-10 inclusive 4 dexamethasone treated and 13 controls started spontaneously.

RESULTS

On the day of T_1 spontaneous onset of labour (SO) did not occur (Fig. 2). On the next day dexamethasone seems not to have exerted any effect but on the following days there was a preponderance of MU in the dexamethasone treated patients. The dexamethasone effect seemed to last up to and including the 6th day. Considering the interval from the 2nd to the 6th day inclusive the number of patients with SO was significantly higher in the dexamethasone group than in the control group ($P < 0.01$). During the next 3 days there was a predominance of SO in the control group.

Twenty three patients in the control group and 12 in the dexamethasone group had IL (Table II). The mean interval from T_1 to induction was 10 days in the control group and 11 days in the dexamethasone group. Four patients in each group were induced before the 7th day after T_1 , thus selection due to induction cannot explain the statistical difference.

Seven patients in the control group were admitted because of ruptured membranes (Table II) and were given an oxytocin infusion because labour did not start within 24 hours. In the dexamethasone group 5 patients were admitted with ruptured membranes in all of them labour started within 12 hours without the aid of oxytocin.

Excluding patients having IL the mean interval from T_1 to SO (T_1 -SO interval) was 11.8 days in the control group and 5.2 in the dexamethasone group the difference being statistically significant ($P < 0.001$).

Pelvic score

In both groups PS increased from T_1 to T_2 (Table III) the score increase of the dexamethasone group being greater than that of the control group. Consistency and effacement were the single factors showing the greatest changes.

The mean PS at T_1 in patients with SO before T_2 is not significantly higher than in those in whom labour had not started 5 days later (Table III).

Uterine activity

OST was performed 193 times in the 120 patients. Even with our modified OST pathological uterine activity may be provoked (Fig. 1).

The uterine activity during T_1 was highest at low oxytocin dosage in patients with SO before T_2 (Table IV). The increase in uterine activity during T_1 was greater among patients in whom labour had not started 5 days later. In the dexamethasone group patients with SO before T_2 had a higher sum of MU for the entire test than the others. This difference is not found in the control group.

Of the 23 control patients with IL 16 had a successful first trial while all the dexamethasone treated women responded favourably to the first trial (Table V). The interval from T_1 to the first trial of IL was shorter in the dexamethasone group and the sum of MU and PS at T_1 was lower in the dexamethasone group than among the controls.

Table II Indications for IL

	Control group n=64	Dexamethasone group n=56
Signs of fetal asphyxia		
Meconium stained amniotic fluid		
Declining placental function	2	2
Oligohydramnios	1	
Abnormal oxytocin challenge test		1
Large fetus	5	3
Primary rupture of membranes	7	0
Proteinuria post term pregnancy	1	2
Troublesome pelvic relaxation	3	1
Maternal desire to have the pregnancy terminated	4	3
Total number	23	12
Mean interval from T_1 to IL days	9.9	8.9*

* Mean interval from T_1 to IL 7.7 days

* Difference between the groups not significant

Table III Mean of pelvic score at T_1 and T_2

	No of patients	T_1	T_2	Increase (%)
Control group				
Nulliparae				
SO after T_1	25	3.3	4.9	54
SO before T_1	15	3.0		
Multiparae				
SO after T_1	20	3.8	5.6	49
SO before T_1	4	4.5		
Dexamethasone group				
Nulliparae				
SO after T_1	14	3.4	6.3	84
SO before T_1	15	3.3		
Multiparae				
SO after T_1	16	3.4	6.8	100
SO before T_1	11	4.4		

The differences between the control and dexamethasone groups give $P < 0.001$

Weight of placenta

Mean placental weight in the control group was significantly higher than in the dexamethasone group (Table VI). As the babies of the nulliparae had a higher birth weight in the control group than in the dexamethasone group (Table I) the placento fetal quotient (placental weight - newborn weight) $\times 100$ was calculated and found to be significantly higher in the control group than in the dexamethasone group (Table VI). The fall in the placento fetal quotient in the treatment group was related to the treatment period (Fig. 3).

Predictive power of OST and PS

Bishop found a correlation between PS and the interval from assessment of the score to the spontaneous onset of labour (1). We correlated the T_1 -SO interval to PS and sum of MU at T_1 for each group and both groups together. The correlation coefficients were less than 0.3. PS and the sum of MU at T_1 were combined in a scoring system ranging from 0 to 37. There was no correlation between this combined score and the T_1 -SO interval. In several cases with a relatively high uterine activity which was also regular and increasing during the OST (as in Fig. 4) labour was seen to start within a few days, so the difference between the mean MU for the two last 10 min periods and the mean uterine activity for the entire OST was calculated. It was not correlated to the T_1 -SO interval. This is in

accordance with the results shown in Table IV. The increase in MU during T_1 is no greater among those showing spontaneous onset of labour before T_2 than among the others.

The standard deviations and coefficients of variation were also calculated. They did not show any correlation with the T_1 -SO interval.

Other findings

The pressure curve of the partonometer may give some additional information. In patients reacting as shown in Fig. 1 great care should be taken in the use of oxytocin. In most cases slight irregularities tend to become normal with increasing dose of oxytocin. If the curve suggests multiple pacemaker areas as in Fig. 5 the labour may be complicated by dystocia affecting the fetus.

Patients having an almost flat contraction curve seldom react favourably to artificial induction during the following week, even if they are post term.

There were 12 operative deliveries in the control group and 9 in the dexamethasone group. Excluding these patients the mean duration of labour was equal in the two groups.

None of the mothers developed arterial hypertension, nor did we recognize any case of intrauterine foetal death. There was no difference in 1 and 5 minute Apgar score between the groups (Table I).

DISCUSSION

The role of corticosteroids in the evolution of human uterine activity

It has been doubted whether corticosteroids can induce labour in women (9, 13). The increased rate

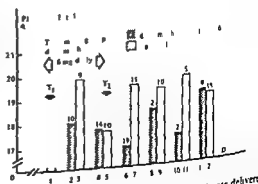


Fig. 3 Mean placento-fetal quotient of patients delivered during each time interval. Numbers on top of bars denote numbers of patients delivered during each interval.

Table IV Mean uterine activity at each dose level of oxytocin during OST

	No of patients	Uterine activity MU											Sum of MU	Increase in sum of MU from T ₁ to T ₂ (%)	
		T ₁						T ₂							
		2	4	6	8	10	12	2	4	6	8	10			12
Control group															
Nulliparae															
SO after T ₁	25	10	13	18	12	25	25	112	11	15	15	22	16	30	170
SO before T ₁	15	13	17	12	15	18	19	94							7
Multiparae															
SO after T ₁	20	9	15	21	24	35	32	135	17	23	38	33	37	39	186
SO before T ₁	4	16	18	21	25	31	27	137							24
Dexamethasone group															
Nulliparae															
SO after T ₁	14	8	10	17	15	22	15	82	17	21	28	32	29	33	161
SO before T ₁	15	17	12	20	25	17	29	119							96
Multiparae															
SO after T ₁	16	3	5	10	7	14	18	57	16	20	26	26	21	30	139
SO before T ₁	11	19	24	22	39	41	48	194							142

spontaneous onset of labour after dexamethasone and the shortened T₁-SO interval in dexamethasone treated patients as found in this study show that corticosteroids do accelerate the evolution of uterine contractions. Mati et al (15) reached the same conclusion when giving betamethasone into the amniotic cavity. Their patients were post-term as were ours. Gennser et al (9) treating 34 term women with betamethasone and comparing them with 17 controls found that the corticosteroid did not influence the duration of

gesterone level (7). They explain the findings as a result of glucocorticoid stimulation of 17 α hydroxylase activity leading to conversion of progesterone to 17 α 20 α -dihydroxypregn-4-en-3-one.

Placental weight

Male placentas are slightly lighter than female ones (12). There was a preponderance of boys in the control group (Table I). Difference in sex ratio therefore cannot explain the low placental weight in the dexamethasone group nor can differing maternal height or weight (Table VI). The placento-fetal quotient in the control group is identical with that reported by Hytten (12). The apparent reversible fall of the placento-fetal quotient (Fig. 3) does not

It seems that in women corticosteroids cannot initiate labour on their own but that one or more other prerequisites must be fulfilled. However the ability of dexamethasone to induce labour is low and its oral administration in the doses used here cannot be a substitute for oxytocin in the induction of labour.

In threatened premature labour the plasma progesterone level is lower if the cause is increased primary uterine activity than if it is secondary to cervical incompetence (6). Scommegna et al found decreased urinary pregnandiol excretion after dexamethasone therapy in pregnant women (21).

Recently Flint et al have demonstrated that in the near term ewe dexamethasone given to the fetus increases maternal and fetal plasma 17 α 20 α -dihydroxypregn-4-en-3-one. Simultaneously they found a decrease in the maternal plasma pro-

Table V Number of patients responding to first trial of IL and mean T₁-SO interval

	No of patients	No of patients with successful IL	T ₁ -SO interval days
Control group			
Nulliparae	11	8	11.1
Multiparae	12	8	9.7
Dexamethasone group			
Nulliparae	5	5	10.2
Multiparae	7	7	8.0

Table VI Mean weight of placenta placento fetal quotient and maternal weight and height

	Placenta (g)	Placental weight newborn weight 100	Maternal Height (cm)	Weight (kg)
Control group	720	19.1	166.1	74.8
S.E.M.	16.2	0.37		
Dexamethasone group	654	17.8	166.9	75.2
S.E.M.	18.0	0.36		

The difference between the groups is significant regarding placental weight and placento-fetal quotient

necessarily imply a causal relationship with the reversible decrease in pregnanediol (21) and estrone excretion (18) after corticosteroid treatment. Corticosteroids may reduce the placental weight in some patients who start in labour and reveal a reduced placento fetal quotient; while those not responding to corticosteroids (far right columns Fig 5) maintain an unchanged placental weight.

Fetal consequences of maternal corticosteroid treatment

We were unable to find any detrimental effect in the babies after dexamethasone treatment as measured by Apgar score (Table I) or observation in the neonatal period by an experienced pediatrician.

Brown et al. (2) did not find any harmful effects from corticosteroids given continuously or for a short period during the pregnancy. However, Warrell & Taylor (23) reported 8 stillbirths and 9 fetuses having been at risk during pregnancy or delivery among 34 women treated continuously during pregnancy with prednisolone. Failure of placental function seemed to be the common cause, but severe maternal disease (bronchial asthma, lupus erythematosus, arterial hypertension) or complications of pregnancy (pre-eclampsia, twins) failed in

duction) may have contributed significantly to the results.

During pregnancy and especially during labour, maternal plasma cortisol levels are increased. A concomitant increase is found in cord plasma (19). Nwosu et al. (17) found that post-term postmature babies had lower morning plasma cortisol levels than term or post-term (but not post-mature) babies. In another study they found that the post-mature babies with low morning cortisol plasma had the same cord plasma cortisol level as the control. However, the post-mature babies lacked the ability to increase the plasma cortisol levels in response to distress during labour, as did vaginally delivered babies at term (16). The results indicate a relative adrenocortical insufficiency in the post-mature fetus. From this point of view, corticosteroid therapy may be a more physiological approach than repeated induction trials with oxytocin or prostaglandins. However, the influence of

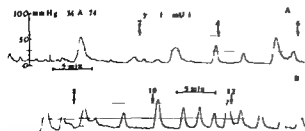


Fig 4 Gravida II para I 6 days past term PS 7 B continuation of A. Uterine activity is coordinated and increases with increasing doses of oxytocin.

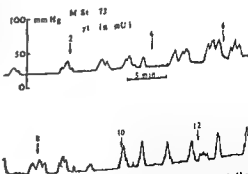


Fig 5 Gravida II para 0 5 days past term PS 3 H. Increased uterine activity improving with increasing doses of oxytocin. Admitted 3 days later with established labour. Administration of amniocentesis and oxytocin resulted in contractions with Caesarean.

corticosteroids on placental weight warrants further studies on the corticosteroid effect on placental function in prolonged pregnancy

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DaktarTM vaginalkram

Nytt antimykotikum för candida- vaginiter/vulviter



- Fungicid effekt
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i cirka 90% oavsett predisponerande faktorer
som diabetes, graviditet, samtidig p-piller- eller antibiotikabehandling
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2-3 dagar
- Olja i vattenemulsion – avtvattbar, färgar ej hud eller kläder

Fass-text

DAKTARTM

Vaginalkram 2^{gr}

Bredspektrumantimykotikum för gynekologiskt bruk

Deklaration

100 g kram innehåller

Miconazol nitrat 2 g

Tefose 63[®] Labrafil M 1944 CS[®] paraffinum liquidum acidum benzoicum butylhydroxanisolum

et aqua purificata g/g

Egenskaper DAKTAR vaginalkram innehåller som aktiv substans miconazol, ett imidazolidinvat. Miconazol har såväl in vitro som in vivo fungicid effekt mot flertalet patogena svampar.

DAKTAR resorberas i mycket ringa grad vid lokal applikation.

DAKTAR har goda kosmetiska egenskaper och innehåller ej lanolin eller parabener. Ett fall av sensibilisering har rapporterats. Krämen färgar ej kläderna och kan avtäckas med tvål och vatten.

Klinik DAKTAR är effektiv vid vaginiter orsakade av Candida spp. och andra svampar.

ter. Såväl mykologisk som klinisk utlakning har konstaterats i ca 90% av de behandlade fallen. Vid behandling med DAKTAR erhålles en snabbt insättande effekt med snabb lindring av symtom som sveda, klåda och flör. Behandling med DAKTAR sänker förhöjda vaginala pH-värden vilket har en gynnsam effekt på den naturliga bakterieflorans tillväxt. DAKTAR lämpar sig väl för behandling av symtomlös mykosit hos gravida som profylax mot oral mykosit hos det nyfödda barnet.

Indikationer Vaginiter och vulviter orsakade av jästsvampar.

Biverkningar I sällsynta fall (mindre än 1%) har lätt lokal irritation rapporterats.

Dosering En fylld applikator (= 5 g kram = 100 mg miconazol) töms djupt i slidan vid sänggåendet. Behandlingen upprepas dagligen i 14 dagar. Det är viktigt för ett gott behandlingsresultat att patienten genomför hela behandlingen. Inget uppehåll behöver göras under menstruation. Vid graviditet bör applikatorn tömmas långsamt och mindre djupt i slidan. Applikator och bruksanvisning medföljer varje förpackning.

Förpackningar Tub à 78 g med applikator.



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THE IMMEDIATE EFFECT OF A β ADRENERGIC AGONIST (SALBUTAMOL) ON CARBOHYDRATE AND LIPID METABOLISM DURING THE THIRD TRIMESTER OF PREGNANCY

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Abstract The responses of plasma insulin and the C-peptide of proinsulin glucose lactate free fatty acids (FFA) glycerol D- β hydroxybutyrate and alanine to a β_2 -adrenergic agonist (salbutamol) were determined in 5 patients during the last trimester of pregnancy before labor. Salbutamol was given as an infusion in the same dosage as is used to inhibit uterine contractions in cases of premature labor and in obstetric emergencies. The infusion of salbutamol was given for 45 min accompanied by repeated sampling of arterial blood. All patients developed moderate tachycardia and exhibited metabolic effects following salbutamol infusion implying marked increases in plasma levels of insulin C-peptide glucose and lactate. The increased rate of lipolysis was evident from the rises of FFA glycerol and D- β hydroxybutyrate. Plasma levels of alanine declined possibly due to stimulation of gluconeogenesis. It is unlikely that these acute maternal metabolic changes would have significant adverse effects on the fetus.

In the 1970s there is an increasing use of adrenergic agonists with β_2 -rather than β_1 receptor affinity such as terbutaline salbutamol and fenoterol. The efficiency with which these drugs induce uterine relaxation has been well documented (1 2 3 4). Cardiac and vascular side effects have been assessed by the same authors. Little attention has however been paid to the metabolic effects on the pregnant women or the fetus. The aim of the present study has been to determine some of the maternal metabolic alterations occurring during intravenous infusion of salbutamol in doses compatible with those commonly applied in obstetrical practice.

MATERIAL AND METHODS

Five patients with various complicating disorders were studied during the 35th to 38th week of pregnancy (Table 1). The investigations were performed with the approval of the regional ethical committee. All patients had body weight and height within normal limits. None of the patients had uterine contractions and they were all studied after an overnight fast. During the investigation the women were in a supine position with the bed tilted slightly (about 15°) towards the left side. Salbutamol in 0.15% NaCl solution was infused into a brachial vein at a rate of 15 µg/min. Heart rate was recorded continuously via a cardiotelemetry triggered by the pulse wave variations in the brachial artery. Blood samples were drawn from the arterial catheter before and during the infusion of salbutamol. Blood samples were collected in heparinized tubes. An aliquot was precipitated immediately in ice-cold 0.6 M perchloric acid for later analysis of lactate (5) while the rest of the sample was stored in ice-cold water. Plasma was separated within one hour and was analysed for glucose (glucose oxidase AB Kabi Stockholm Sweden) free fatty acids (FFA) (6) glycerol (7) D- β hydroxybutyrate (8) alanine according to a microfluorimetric method adapted after Williamson et al (9) insulin (Phadebas® AB Pharmacia Uppsala Sweden) and C-peptide of proinsulin (10). All analyses were done in duplicate.

RESULTS

Salbutamol infusion resulted in moderate maternal tachycardia (Fig 1). The heart rate increased from base line values of 93 ± 6 (S.E.M.) to a maximum of 134 ± 4 beats/min. Some patients experienced palpitation and slight tremor of the hands during the infusion of salbutamol. Parturition occurred 1-4

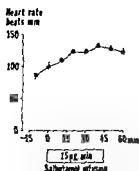


Fig 1 Response of maternal heart rate to intravenous salbutamol infusion (mean values and S.E.M.)

weeks after the investigation with no perinatal complications

The concentrations of metabolites insulin and C peptide in blood and plasma showed significant alterations following the administration of salbutamol (Fig 2 and 3). Thus the concentrations of glucose, insulin, C peptide, FFA, glycerol, D-β-hydroxybutyrate were already significantly elevated at 15 min, lactate at 30 min and alanine showed a significant drop at 30 min (paired *t* test). The mean molar ratios between C peptide and insulin were 6.7 and 6.0 respectively during the control period and declined to 3.8 at 15 min, 3.9 at 30 min, 4.6 at 45 min and 4.5 at 60 min following the infusion of salbutamol.

Statistically significant correlations ($p < 0.001$) were found between the following parameters: C peptide and insulin ($r = 0.93$), glucose and insulin ($r = 0.89$), FFA and glycerol ($r = 0.64$) and FFA and D-β-hydroxybutyrate ($r = 0.68$).

DISCUSSION

The doses of salbutamol used in the present study were in the same range as those given to pregnant

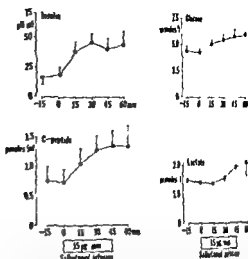


Fig 2 Responses of plasma insulin, C-peptide, glucose and blood lactate to intravenous salbutamol infusion (mean values and S.E.M.)

women by the same route of administration with the aim of inhibiting contractions in cases of premature labor (2) and in obstetric emergencies (11). All patients developed moderate tachycardia in agreement with earlier reports (2, 3, 4) but none of them showed evidence of serious cardiovascular side-effects.

The infusion of salbutamol induced a prompt increase in plasma concentrations of glycerol and FFA (Fig 3) indicating an enhanced rate of lipolysis.

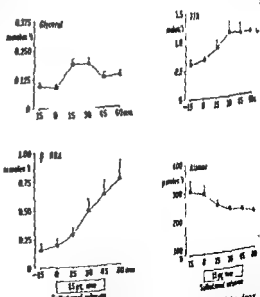


Fig 3 Responses of plasma glycerol, FFA, D-β-hydroxybutyrate and alanine to intravenous salbutamol infusion (mean values and S.E.M.)

Table 1 Clinical data on the pregnant patients

Patient	Age	Parity	Gestation at test (weeks)	Complications during pregnancy
AI	18	0	37	Susp. placenta praevia
BD	32	1	38	Susp. IUGR*
BP	23	0	37	Susp. IUGR
BB	18	1	35	Proteinuria
MB	37	0	37	Susp. placenta praevia

IUGR=intrauterine growth retardation

mobilization. This is in agreement with the β_2 receptor stimulating property assigned to salbutamol. The combination of an increased rate of lipid mobilization and marked increments in plasma insulin levels has also been observed in non-pregnant subjects following intravenous administration of either salbutamol (12) or isoproterenol, a β_1 and β_2 -receptor stimulating agent (13). The parallel increase in plasma levels of insulin and C-peptide—the connecting polypeptide segment of proinsulin—after salbutamol infusion (Fig. 2) suggests enhanced release from the pancreas. The calculated molar ratios between C-peptide and insulin in plasma in the pregnant women were similar to those observed in nonpregnant subjects (10). After stimulation of insulin release by salbutamol in pregnant women and by oral glucose in non-pregnant subjects (10) the corresponding ratios decreased in a similar fashion. Previous observations on the effect of salbutamol in dogs (14, 15) and in man (12) indicate that the increased insulin release is caused by direct stimulation of pancreatic β_2 -adrenergic receptors. This interpretation was based on the fact that blood glucose levels either remained unchanged (14, 16) or showed only a slight increase which tended to occur after the decrease in insulin concentration induced by salbutamol (12). In the present study, however, we observed a close correlation in time between the rise in plasma levels of glucose and of insulin. This discrepancy in results might be due to several factors: the pregnant state as such, differences in doses of salbutamol, ways of administration or different timing of the blood sampling.

The elevations of both plasma glucose and blood lactate concentrations induced by salbutamol in the present study could be due in part to an increased rate of glycogenolysis. The rise in glucose concentrations might also be explained by increased gluconeogenesis, a concept supported by the finding of decreased plasma levels of alanine. The steady increase in D- β -hydroxybutyrate concentration suggests an increased rate of ketogenesis. An increased production of ketone bodies by the liver would be favoured by the accelerated mobilization of free fatty acids and perhaps also by the increased plasma levels that are known to occur following β -adrenergic stimulation (17).

Infusion of salbutamol in doses used in obstetrical practice induced a series of acute changes in the metabolism of the pregnant woman. It is unlike

ly however that these maternal metabolic changes caused by the salbutamol infusion during a short period would have significant adverse effects on the fetus. It has been shown that β receptor stimulating agents are transferred across the primate placenta almost instantaneously and the same group of investigators have demonstrated that an acute effect of the administration to the mother of β receptor stimulating drugs is represented by a diminution of fetal arterial pO_2 (18). In the clinical situation of unwanted labor, especially obstetrical emergency, however, the possible adverse metabolic and circulatory effects on the fetus of β -adrenergic agonists must be balanced against the benefit of efficient inhibition of uterine contractions.

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OUTCOME OF PREGNANCY IN THE PRESENCE OF INTRAUTERINE DEVICE

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Abstract The outcome of 196 pregnancies with Lippes loop in situ was studied. In 102 cases with inaccessible IUD the IUD was left in place. Ninety-four women had the IUD removed when the thread was still visible. The incidence of spontaneous abortion and premature delivery was 4.1% in the group of 102 and was significantly higher than in those women whose loop was removed. No serious complications occurred in any of the cases. The study suggested that the IUD should be removed early in pregnancy when the string is still accessible.

The occurrence of pregnancy following IUD insertion is a distressing problem to both physician and patient, especially in countries where induced abortion is illegal. This study reports an investigation into the outcome of pregnancy with IUD in situ and the effects of IUD on mother and fetus.

MATERIALS AND METHODS

During the period 1968-1975, 210 women were diagnosed as pregnant with IUD in situ at the family planning clinic of Surin Hospital. In cases where the IUD thread was still visible, the IUD was removed by gently pulling the thread. If the thread was inaccessible, no attempt was made either to remove or locate the IUD. All cases were closely followed up in antenatal clinic until pregnancy terminated. In those cases whose IUD was not removed, an X-ray of abdomen was used to locate the IUD if it was not found during abortion or delivery. Cases of IUD expulsion before pregnancy, extrauterine pregnancy and extrauterine pregnancy were excluded from the study.

RESULTS

Of 210 cases followed, there were 4 tubal pregnancies, 1 ovarian pregnancy, 2 extrauterine IUDs and 7 cases of proven expulsion of IUD before pregnancy (the IUD was not removed and was not removed during or after pregnancy termination).

The remaining 196 were confirmed cases of intrauterine pregnancy with IUD in situ. Of these 196 women, 102 had an inaccessible IUD which was left in the uterus (group A). The IUD was removed in 94 women with visible threads (group B). As shown in Table I, the accessibility of the thread depended mainly on the length of gestation. Of cases seen at 8 weeks pregnancy or less, 98.8% had a visible thread. This contrasted with 19.1% and 2.1% of the cases seen after a 9-12 weeks pregnancy and after 12 weeks pregnancy respectively who had a visible thread.

All patients were using the Lippes loop and size D loop was used in the majority of both groups.

Table I Maternity and visible IUD thread

Maternity	Group A Thread not visible IUD left in situ		Group B Visible thread IUD removed		Total	
	No	%	No	%	No	%
≤8	1	1.2	80	98.8	81	100
9-12	55	80.9	13	19.1	68	100
≥13	46	97.9	1	2.1	47	100
Total	102	52.0	94	48.0	196	100

Table II Lippes Loop size

Lippes Loop (size)	Group A		Group B		Total	
	No	%	No	%	No	%
B	9	8.8	3	3.2	12	6.1
C	28	27.5	31	33.0	59	30.1
D	65	63.7	60	63.8	125	63.8
Total	102	100.0	94	100.0	196	100.0

Table III Duration of use

Duration of use (m)	Group A		Group B		Total	
	No	%	No	%	No	%
≤6	21	20.6	5	6.4	27	13.8
7-12	13	12.7	21	22.3	34	17.3
13-18	17	16.7	16	17.0	33	16.8
19-24	14	13.7	14	14.9	28	14.3
>24	37	36.3	37	39.4	74	37.8
Total	102	100.0	94	100.0	196	100.0

(Table II) The duration of use up to the time of conception ranged from 3 to 38 months (Table III). The pregnancy rate cannot be estimated as several cases were referred from other hospitals or health centers.

The age groups, parity and number of previous abortions are shown in Tables IV, V and VI respectively. The incidence of abortion was 7.6% and 5.1% of total pregnancies in group A and group B respectively.

OUTCOME OF PREGNANCY

In 102 women whose IUD was not removed, spontaneous expulsion at 3-4 months gestation occurred in 2 women (2%) whose pregnancy then went to term without complication. Ninety-eight women (96%) had spontaneous expulsion of the IUD during abortion or delivery. Two women (2%) retained the IUD in the uterus after delivery (Table VII).

As shown in Table VIII, when the IUD was not removed, 45 women (44.1%) aborted spontaneously within the first 12 weeks and 13 women (12.7%) after 12 weeks of pregnancy. Two women (2%) had fetal death and delivered prematurely.

Table IV Age group

Age group	Group A		Group B		Total	
	No	%	No	%	No	%
≤24	32	31.4	26	27.7	58	29.6
25-29	37	36.3	30	31.9	67	34.2
30-34	22	21.6	21	22.3	43	21.9
35-39	8	7.8	14	14.9	22	11.2
40-45	3	2.9	3	3.2	6	3.1
Total	102	100.0	94	100.0	196	100.0

Table V Parity

Parity	Group A		Group B		Total	
	No	%	No	%	No	%
1	9	8.8	14	14.9	23	11.7
2	24	23.5	30	31.9	54	27.5
3	15	14.7	11	11.7	26	13.2
4	18	17.6	6	6.4	24	12.1
5	36	35.3	33	35.1	69	34.9
Total	102	100.0	94	100.0	196	100.0

Table VI Previous abortion

Abortion	Group A		Group B		Total	
	No	%	No	%	No	%
0	82	80.4	81	86.1	163	82.8
1	13	12.7	10	10.6	23	11.7
2	5	4.9	3	3.1	8	4.1
3	2	2.0	-	-	2	1.0
Total	102	100.0	94	100.0	196	100.0

Premature live birth occurred in 9 women (8.8%). There was one microphthalmos full term fetus.

Among 94 women whose IUD was removed, there were 21 cases (22.3%) of spontaneous abortion within the first 12 weeks of pregnancy within 3 weeks after removal of IUD (Table V). Removal of the IUD might be the cause of abortion in some of these cases. Three women (3%) spontaneously aborted after 12 weeks of pregnancy and over one month after removal. Only 4 women (4.3%) delivered prematurely. There were no fetal deaths among full term and premature infants.

The incidence of abortion and premature labor in group A proved by the Z test to be significantly higher than in group B ($P < 0.05$). No sepsis or other serious complications were observed in both groups.

Table VII IUD status in group A

Status	No	%
Spontaneous expulsion at 3-4 months pregnant	2	2
Spontaneous expulsion during delivery or abortion or removed during curettage	98	96
Retained in uterus after delivery	2	2
Total	102	100

Pregnancy

Table VIII Pregnancy outcome

Pregnancy outcome	Group A		Group B		Total	
	No	%	No	%	No	%
Full term Live birth	31	30.4	62	63.9	93	47.4
Premature Live birth	9 ^a	8.8	4	4.3	13	6.6
Intrauterine death	2	2.0	-	-	2	1.0
Spontaneous abortion						
≤17 weeks pregnant	45	44.1	21	22.3	66	33.7
≥17 weeks pregnant	13	12.7	3	3.2	16	8.2
Induced abortion	2	2.0	4	4.3	6	3.1
Total	102	100.0	94	100.0	196	100.0

^a One microphthalmos^b Including 1 neonatal death from prematurity

DISCUSSION

The results of this study show that the incidence of premature delivery and spontaneous abortion in pregnant women with retained IUD is significantly higher than in those in whom the IUD was removed. This finding was similar to those reported previously (1, 2, 3). Thompson (4) however believed that if the IUD string is visible the IUD is down in the lower uterine segment or in the endocervical canal and that the pregnancy is probably going to survive whether the IUD is removed or not. Evidence in the present study on the contrary shows that the visibility of the thread depends mainly on the gestational period. Before 8 weeks of pregnancy almost all cases will have visible threads which will become inaccessible when pregnancy is advanced.

There were 2 intrauterine fetal deaths which occurred during 28-32 weeks of pregnancy in the unremoved group. Whether the IUD was the cause of death in these 2 cases or not is uncertain. Fetal

death might be due to subclinical premature separation of the placenta provoked by the IUD if the placental attachment was above the IUD (5).

There have been several reports concerning haemorrhage and sepsis complicating pregnancies with IUD mostly with Dalkon Shield and to a lesser extent with Lippes loop or other IUDs (4, 5, 6, 7). Amniotic fluid embolism associated with pregnancy with the IUD in situ has also been reported (8, 9). To avoid possible serious complications Dreishpoon (7) recommended that early abortion be performed when pregnancy is associated with an IUD however in this study there were no serious complications observed in either group of patients. Therapeutic abortion therefore cannot yet be justified on grounds of protecting the mother's health in countries where abortion is still illegal.

In conclusion based on the results of this study when pregnancy occurred with IUD in situ the IUD should be removed as soon as possible if the string is still visible. If the string is inaccessible the IUD should be left undisturbed and the patient closely followed up.

ACKNOWLEDGEMENT

We wish to express our sincere thanks to Professor Pradhand Anyantr and Mr Anthony Bennett for their help as to all personnel of Surraj Family Planning Research Unit who assisted the authors in this study.

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Table IX Time interval between removal and abortion

Interval (days)	No	% of total cases removed (94)
≤7	10	10.6
8-14	7	7.4
15-21	4	4.3
22-28	-	-
29-35	3	3.2
Total	24	

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SCINTIGRAPHIC STUDIES OF UTERINE AND PLACENTAL GROWTH AND PLACENTAL MIGRATION DURING PREGNANCY

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fetal Placental scintigraphy with ^{125}In (Indium) administered with cervical marking with a shielded ^{57}Co radioactive source was used to study uterine and placental growth in human pregnancy and placental local migration in a total of 176 patients. Uterine length measurements can be used for selecting growth retarded pregnancies. There was an approximately constant ratio between placental diameter and uterine length (0.68 ± 0.03). When the placenta was located on the ventral uterine wall no migration occurred in 61%. The corresponding percentage for low implantation when the placenta was located on the dorsal uterine wall was 30%. The difference was highly significant. Placental migration was studied in 20 cases. Significant migration occurred in 11 cases. The internal margin closest to the internal cervical os moved outwards about 3 cm on average.

fetal representation of uterine growth has been shown to be of value in monitoring at risk patients (1-4) and is therefore a means of reducing perinatal mortality and morbidity. For this reason it seemed of interest to measure the size of the uterus and the location in situ. It is investigated for placental localization. Because of the distension of the lower uterine segment it was suggested (5) that placentas located in the lower uterine segment might migrate away from the internal cervical os during the course of pregnancy. A hypothesis which was later confirmed.

The present study describes the distribution of the placental site within the uterus, the magnitude of placental migration and growth curves of the fetus and the placenta using scintigraphic techniques.

MATERIAL AND METHODS

A polaroid camera PHi/Gamma III HP (Nuclear Chicago) with a diverging collimator was used. Every patient was

examined from a frontal and a lateral projection (Fig. 1). The oscilloscopic view was photographed by means of a polaroid camera after intravenous injection of 1 mCi ^{125}In . The cervical internal os was marked with a ^{57}Co radioactive source as described earlier (8). The length of the uterus was measured in the sagittal plane (Fig. 2). The maximal diameter of the placenta and the distance between the caudal margin of the placenta and the cervical marking was measured. The center of the placenta with respect to location in either of the two upper or the two lower quadrants in the frontal projection and on the ventral or dorsal wall in the lateral projection was recorded. As the collimator was of a diverging type it was necessary to use a correction factor in order to convert the figures obtained from the polaroid picture into centimeters. The correction factor varied in relation to the distance (Fig. 3). The average distance A in Fig. 1 is the distance from the collimator to a plane in the patient through the ^{57}Co -marker was about 20 cm. A variation of ± 1 cm from the average distance implies an error in the correction factor of $\pm 3\%$.

176 patients were examined. The reasons for investigation were vaginal bleeding 117, abnormal presentation 17, examination prior to amniocentesis 15, abdominal pain 10, retarded fetal growth 5, and miscellaneous 12. 19 patients were examined on two occasions and one patient on three occasions during the course of the same pregnancy. The growth curves were constructed from 136

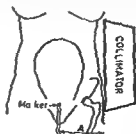


Fig. 1 Variation in distance between collimator and cervical marker in the lateral projection. Gestation in weeks 19-42. $A = 20.7 \pm 2.8$ (S.D.) cm. $N = 87$.

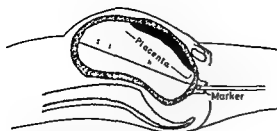


Fig 2 Method of measuring uterine sagittal length and largest placental diameter

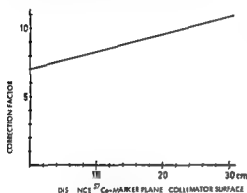


Fig 3 Correction factor in relation to distance between radionuclide and collimator

results obtained in patients delivered of a normal for date infant, i.e. within ± 2 S.D. of the mean for weight and length (1).

RESULTS

Placental localization The distribution of dorsally and ventrally situated placentas in the four quadrants of the uterus is shown in Fig 4. With 30 located on the ventral wall and 96 on the dorsal wall there was no tendency towards favouring of either

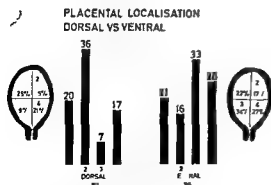


Fig 4 Distribution of placental sites in the dorsal and ventral parts of the uterus



Fig 5 Distribution of placental sites in 4 quadrant frontal projection

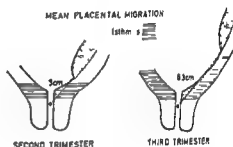


Fig 6 Mean placental migration in 11 patients

location. In the total number studied 51% placentas were situated in the upper quadrant, 49% in the lower quadrants (Fig 5). Of the ally situated placentas 61% were located lower part of the uterus while only 30% dorsal placentas were in this situation the ence is statistically significant ($p < 0.001$).

Placental relationship to the internal cervical os Patients ($n=20$) examined on two or more occasions during the course of the same pregnancy were

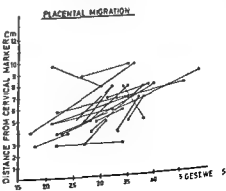


Fig 7 Placental migration in the individual patients relation to gestation

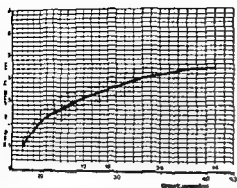


Fig 8 Uterine length in the sagittal plane (Mean \pm 1 S D) in relation to length of gestation

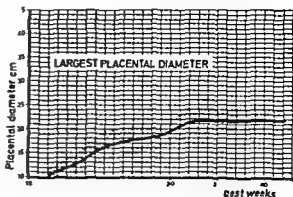


Fig 9 Largest placental diameter (Mean \pm 1 S D) in relation to length of gestation

measured at intervals varying from 3 to 19 weeks. In 18 of these repeated examinations we noticed an increase in the distance from the placental margin to the cervical marking. In 11 examinations the distance increased more than 1 cm and on average increased by 3.3 cm (Fig 6). In one the distance was unchanged and in two it was decreased. In the two latter cases the distances at the first examination were 8 and 10 cm respectively (Fig 7).

Uterine and placental growth curves. Fig 8 shows the growth curve of the uterus obtained by scintigraphic measurement of its sagittal length. The growth curve of the placenta has a slightly different shape (Fig 9). The ratio between placental diameter and uterine length was fairly constant throughout pregnancy (0.68 ± 0.03).

DISCUSSION

The indication for a scintigraphic examination in this study was a pregnancy complicated by one or more factors. Vaginal bleeding was the reason for investigation in no less than 67% of the patients. This accounts for the fairly high frequency of placenta praevia in the lower segment of the uterus (49%). A similar pattern has been described in another study (7). This study has shown that there is a tendency for the placenta to be implanted either in the upper dorsal part or in the lower ventral part of the uterus. The cause of this is unknown.

The reason why the placentas implanted in the lower part of the uterus migrated away from the cervix with advancing gestational age is probably due to the continuous enlargement of the isthmus during pregnancy. Thus a placenta praevia

diagnosed in the second trimester can develop into a marginal or low implanted placenta in the third trimester. Consequently there is a progression towards a lower obstetrical risk group. It is therefore reasonable to reexamine patients with placenta praevia diagnosed in early pregnancy.

The uterine growth curve appears to be of value in assessing fetal growth retardation. Three out of four small for dates infants had uterine length measurements well below mean -2 S D. This is not unexpected since there is a close correlation between the length of the uterus and the fetal crown-rump length (CR). Calculation from available data on CR (7) shows that the CR is 80% of the uterine length at 26 weeks gestation and at the 35th week of pregnancy is 90%. At 40 weeks both measurements are equal.

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FETO MATERNAL BLEEDING

During Pregnancy and at Delivery

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Abstract Blood samples from pregnant women and from mothers before and after delivery were tested for the occurrence of feto-maternal bleeding (f m b) using Kleihauer acid-elution technique and a counting technique described by Schneider. It was assumed that f m b had occurred when there was a clinically significant difference between the values of hb-F cells per million cells in two blood samples. F m b had occurred in nearly 2/3 of the mothers after pregnancy and delivery. The f m b s were most often less than 0.1 ml and only a few per cent had f m b s between 0.1 and 1 ml. Abortion complicated pregnancies, amniocentesis, pre-eclampsia, caesarean section and other kinds of complicated deliveries increased the risk of especially larger f m b s.

The blood from the fetus is normally separated from the mother's blood by a membrane in the placenta. However, fetal red cells can pass this membrane as shown by Chown (4). Zipursky (26) was the first to use the simple acid elution method described by Kleihauer (11) for the detection of fetal red cells in maternal blood. Since then several investigations of the occurrence of feto-maternal bleeding (f m b) have been performed.

In nearly all the published investigations the occurrence and amount of f m b have been measured on the basis of the number of haemoglobin F containing red cells (hb-F cells) in a single blood sample. However, a better estimate of the occurrence of f m b and exactly when it occurs can be made by counting hb-F cells in blood samples taken at different times. The aim of the present study has been to investigate the occurrence of f m b in connection with various obstetrical conditions, counting hb-F cells in blood samples taken before and after these obstetrical conditions.

METHODS

The hb-F cells in maternal blood were found by Kleihauer (11) acid-elution technique used in a slightly modified way (7). The hb-F cells were counted as described by Schneider (22).

On the basis of the statistical analysis of the accuracy of the counting method, it was decided that two values of hb-F cells/million show a clinically significant difference when one of the values is 0 and the other more than 3, or when one of the values is greater than 0 and the other 50% higher and at least 10 (7). If a clinically significant difference in hb-F cells/million was obtained by counting cells from two different blood samples from the same woman and the higher value was obtained in the second blood sample, it was assumed that f m b had occurred in the period between the withdrawal of the two blood samples.

The volume of the f m b was calculated as described by Kleihauer (10).

MATERIAL

Pregnancy The series consists of 225 normal (23) pregnant women all delivering an ABO compatible child. They were asked to come to the hospital once every month from the third month of pregnancy until delivery. In 337 cases we have succeeded in obtaining 2 blood samples with an interval of 1 month. If there had been an f m b it was assumed to have occurred in the month when the first blood sample was taken. In 24 instances a blood sample was taken in the 9th month of pregnancy and just before labour. Blood samples were also taken from pregnant women with pre-eclampsia.

Abortion Blood samples were taken from 55 women just after spontaneous abortion and from 186 women before and after induced abortion. ABO-incompatible cases were not excluded from these groups because the fetuses were not ABO grouped.

Amniocentesis Blood samples were taken just before and 1-7 days after amniocentesis done in 54 normal pregnant women delivering an ABO compatible child. N

Table I Occurrence of foeto maternal bleedings

F m b (ml)	Pregnancy			Induced abortion	Amniocentesis 3rd trim			Delivery		
	2nd trim	3rd trim	Total		Normal	Comp	Total	Normal	Caesarean section	Other compl
0	139	165	304	154	69	6	75	61	31	11
<0.1	9	18	27	23	3	0	3	6	6	1
0.1-1	3	3	6	5	1	3	4	3	3	2
>1	0	0	0	3	1	2	3	0	4	0
Total	151	186	337	186	74	11	85	70	44	14
	%	%	%	%	%	%	%	%	%	%
0	92	89	90	83	93	55	88	87	70	79
<0.1	6	10	8	12	4	0	4	9	14	7
0.1-1	2	2	2	3	1	27	5	4	7	14
>1	0	0	0	2	1	18	4	0	9	0
Total	100	101	100	100	99	100	101	100	100	100

of the mothers were Rh immunized. All the amniocentesis were done in the third trimester.

Delivery. Blood samples were taken before (less than 24 h before delivery but before second stage pains) and after 70 normal (23) and 58 complicated deliveries. All cases with ABO incompatibility and Rh negative mothers with Rh positive children were excluded (8).

RESULTS

Pregnancy. F m b was found in 8% per month in the 2nd trimester and in 12% per month in the 3rd trimester indicating a slight increase in the tendency to f m b during pregnancy (Table I). F m b in the 6th-9th months was seen in 6 (43%) of 14 samples

from pregnant women with pre-eclampsia. This was significantly ($p=0.0056$) more frequent than in women with a normal pregnancy where it was seen in 10 (9%) of 111 samples taken in the same months of pregnancy.

Abortion. After 55 spontaneous abortions in the 1st trimester hb F cells were found in 35%. This was significantly ($p<0.01$) more frequent than in a control series consisting of normal pregnant women tested in the 1st trimester before induced abortion (Table II). The amounts of hb F cells corresponded to f m b's below 50 μ l.

F m b occurred in 17% of 186 women in connection with induced abortion in the 1st trimester. The f m b was between 0.1 and 2 ml in 4% (Table I). The occurrence of f m b was apparently not dependent upon the method used for the induced abortion (suction curettage, intra amniotic injection or amniotomy).

Amniocentesis. F m b occurred in connection with 6% of 74 normal amniocentesis but in 45% of 11 complicated amniocentesis (blood in the syringe) which is significantly ($p=0.0005$) more frequent than in normal amniocentesis (Table I).

Delivery. After normal deliveries f m b's were found in 13% and the bleeding amounted to more than 0.1 ml in 4%. After caesarean section f m b's were found in 30% of 44 mothers and the bleeding amounted to more than 0.1 ml in 16% which is significantly ($p=0.02$) more frequent than after normal deliveries. After other kinds of complicated deliveries f m b's were found in 21% and the bleeding amounted to more than 0.1 ml in 14% (Table I).

Table II Occurrence of hb F cells per million red cells from maternal blood before and after abortion

b-F s/ million	Spontaneous abortions (After)	Induced abortions	
		Before	After
0	36	160	102
1-2	12	15	33
3-9	7	8	33
10-19	0	2	8
≥ 20	0	1	10
Total	55	186	186
	%	%	%
0	65	86	55
1-2	22	8	18
3-9	13	4	18
10-19	0	1	4
≥ 20	0		5
Total	100	99	100

DISCUSSION

F m b s can easily be detected and measured using the acid-elution method (10). If a sufficiently large number of red cells from maternal blood are looked upon (more than about 1 million) it is possible to detect bleedings as small as 25 μ l (5 hb-F cells per million maternal cells). The result is most unambiguously indicated as hb-F cells per maternal cells or as f m b. Cases with blood group incompatibility between mother and fetus should be excluded from the study. Such incompatibilities will give a greatly reduced survival time of the fetal red cells in the maternal circulation. Therefore in these cases a f m b will be estimated to be too small or even not be detected at all (6). In the following only cases satisfying these criteria will be discussed.

Pregnancy. F m b is theoretically possible from the 4th week of pregnancy when the fetal and the maternal blood circulations in the placenta have been formed. However occurrence of hb-F cells in maternal blood from mothers in the 1st trimester cannot immediately be taken as evidence for a f m b because pregnant women in the 1st trimester have an increased production of haemoglobin F (12, 20). The same is not the case in the 2nd and the 3rd trimester.

In the present investigation f m b could be found in 8% per month in the 2nd trimester and in 12% per month in the 3rd trimester indicating a slight increase in the tendency to f m b during pregnancy. The f m b s were mainly small bleedings only amounting to 0.1-1 ml. The fetal red cells will survive in the mother for 3-4 months. Therefore hb-F cells will be found in an increasing number of mothers during pregnancy. On the basis of the occurrence of f m b s per month one would expect that a little less than 44% (8+12+12+12% in the 6th, 7th, 8th and 9th month and some of the f m b s will occur in the same woman) will have hb-F cells in their blood just before delivery. Investigation of blood samples from 150 women in the 9th month showed that hb-F cells were found in 39% of the samples (6).

In women with pre-eclampsia a significantly higher tendency to f m b was found in the 3rd trimester. This is in accordance with previously published investigations (1, 21, 27).

Abortion. After the 1st trimester spontaneous abortions a significantly larger number of women with hb-F cells was found than in normal pregnant women in the same period of pregnancy. In none of

the cases was it possible to detect hb-F cells corresponding to a f m b of more than 0.1 ml. The same result was found in other investigations where the maternal included a control group of normal pregnant women in the same period of pregnancy (1, 9, 14, 18).

After induced abortions f m b was found in 17% and in 5% it amounted to between 0.1 and 2 ml. In previously reported investigations f m b was found in 3% and 7% (>0.25 ml) and in 3% and 13% (>0.1 ml) (16, 24, 18, 1). The present series was not large enough to show any significant difference between the occurrence of f m b after induced abortion using different methods.

Amniocentesis. F m b bleeding was found in 6% after a normal amniocentesis and in 45% after a complicated amniocentesis showing the enormous risk of f m b during amniocentesis. Previously described cases have all consisted of Rh immunized women with Rh positive fetuses. Despite this f m b was also found in 8-11% of the women in these series (1, 3, 13, 19, 25, 28).

Delivery. During delivery the membrane that separates the fetal and the maternal circulation is broken. This increases the risk of f m b. After delivery there is also a possibility of f m b by the transperitoneal route through the abdominal cavity (5).

Investigation of samples taken before and after a normal delivery showed that f m b occurred in 13%. None of the bleedings exceeded 1 ml. In previously published series excluding cases of ABO incompatibility an increased amount of hb-F cells was found in 11% (21). Series including complicated deliveries showed increased amounts of hb-F cells in 25%, 28% and 26% (2, 15, 1). In connection with complicated deliveries f m b was found in 30% of 44 cases with caesarean section in the present series. This is significantly more frequent than after normal deliveries. In 9% of the cases there was a f m b of more than 1 ml. After other kinds of complicated deliveries f m b was found in 21% but none of more than 1 ml. Montague (17) found f m b s of more than 0.25 ml in 30% of women with complicated deliveries.

F m b occurs very frequently even in normal pregnancies and deliveries. During the last 6 months of pregnancy it occurs in 10% per month more frequently in the 3rd trimester than in the 2nd trimester and after delivery a further 13% have had a f m b. So before delivery half of the

women have had a f m b and after delivery 2/3. Complicated pregnancies and deliveries increase the risk of f m b. F m b is thus seen in at least 6% after amniocentesis, in 17% after induced abortion, in 43% of mothers with pre eclampsia, in 30% after caesarean section and in 21% after other kinds of complicated deliveries.

The amount of the f m b is normally less than 0.1 ml and in only a few per cent of the cases is it between 0.1 and 1 ml. However, complicated pregnancies and deliveries increase the risk of larger f m b's. F m b's of more than 1 ml were thus only seen after induced abortion, amniocentesis and caesarean section, but it was still not very common.

For the fetus these small f m b's are of no importance. It is only the very seldom occurring large f m b's which involve a risk for the fetus. However, for the mother even these small bleedings will involve a high risk of immunization against fetal antigens.

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A COMPARISON BETWEEN THE LECITHIN/SPHINGOMYELIN RATIO AND THE NILE BLUE SULPHATE TEST IN THE ESTIMATION OF FETAL MATURITY

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Abstract Functional fetal maturity was assessed in 72 patients by measuring in amniotic fluid the lecithin/sphingomyelin ratio and the differential cell count. The correlation between these two tests is presented. Only one fetus with a lecithin/sphingomyelin ratio of 2 or more developed the respiratory distress syndrome after delivery that was the child of a diabetic mother. The technical limitations of the lecithin/sphingomyelin ratio meant that it could not be measured in 12% of samples obtained.

The L/S ratio of amniotic fluid is a measure of fetal pulmonary maturity. It is an index of the concentration of dipalmitoyl lecithin which is the major surface active agent in the mature lungs (9). A baby born before it has the capacity to synthesize sufficient dipalmitoyl lecithin is likely to develop the respiratory distress syndrome (RDS).

The Nile blue sulphate (NBS) test is thought to give a measure of the functional maturity of sebaceous glands (4). Cells from the glands are found in amniotic fluid and the percentage stained orange by the dye increases with gestational age.

This paper compares the L/S ratio to the NBS test and examines their use in the management of a planned delivery.

PATIENTS AND METHODS

The series comprises 77 consecutive patients in whom induction of delivery was contemplated. Seventy-five samples of amniotic fluid were collected by transabdominal amniocentesis.

Each sample was evaluated by both the L/S ratio and the NBS test. The obstetric management of the patients was then conducted with full reference to the results obtained.

Lecithin/sphingomyelin ratio

The L/S ratios were determined by a modification of the method of Gluck & Kulovich (9). Two ml of centrifuged amniotic fluid were submitted to solvent extraction and acetone precipitation. The extracts were chromatographed using the system of Coch and co-workers (2). The spots were visualized by spraying with 10% aqueous ammonium sulphate followed by charring by heating at 250°C for 15 min. The lecithin and sphingomyelin spots were measured in two dimensions and the results were reported as lecithin/sphingomyelin area ratios. By the use of standard dipalmitoyl lecithin and sphingomyelin a linear relationship was found between spot size and phospholipid concentration over the range normally encountered (10-100 µg).

An L/S ratio of 2.0 or greater is said to indicate functional maturity of the fetal lungs. With ratios of less than 1.5 the incidence of significant respiratory distress is 100%. When the L/S ratio is 1.5-2.0 some patients develop respiratory distress but much of it will be mild (7).

Amniotic fluid cytology by the Nile blue sulphate stain

All samples were analysed within 24 hours of being taken and interpreted according to the criteria of Husain & Sinclair (10) and Brosens & Gordon (1) (Table 1).

Clumping of the cells is a technical problem which affects the cell counting as recognized by Brosens & Gordon (1). In one case there was a fall from 15 to 5% orange staining cells in samples taken 2 weeks apart due to this effect.

The neonates

The maturity of all babies was assessed clinically by a member of the medical staff within 24 hours of birth.

RESULTS

The results of the tests on the amniotic fluid samples are shown in Fig. 1. This indicates the correlation between the L/S ratio and the NBS test.

Table 1 *The interpretation of the Nile blue sulphate test*

After Brosens & Gordon 1966

Percentage of cells staining orange	Gestational age (weeks)
0-1	0-34
1-10	34-38
10-50	38-40
>50	>40

The 72 mothers in the series were delivered of 74 babies there being two sets of twins. The mean birthweight was 3.23 kg (7 lb 2 oz) with a standard deviation of 0.4943 and a coefficient of skewness of 0.1697. This result does not differ significantly from the zero coefficient of skewness of a normal distribution ($P < 0.05$) (16) and suggests that the babies represented a normal sample.

Respiratory disorders developed in 3 neonates: one pneumomediastinum, one primary pulmonary atelectasis and one case of RDS.

Case report of the respiratory distress syndrome

This was the second child born to a 25 year old Caucasian mother. The mother is a diabetic who requires insulin only when pregnant to a maximum dose of 40 units per day. The diabetes is controlled by diet alone when she is not pregnant. This pregnancy was otherwise normal. Radiological assessment of fetal maturity at 37 weeks gestation showed a baby of 36-37 weeks maturity. The results of amniocentesis one week later were: L/S ratio 2.5, NBS test 22% orange staining cells = 39-40 weeks maturity.

Labour was induced by artificial rupture of membranes at 39 weeks on the basis of these results. The labour did not progress well and monitoring of the fetal heart rate fetal distress.

A baby weighing 3640 g (8 lb) was delivered by Caesarean section with the umbilical cord once round its neck. He was resuscitated at birth with the aid of endotracheal intubation. The Apgar scores were 2 at 1 min, 4 at 5 min and 8 at 15 min. Regular respirations were established at 14 min and the baby was extubated at 21 min after delivery.

The respiratory rate was 60 per min at 6 hours after birth and a chest X-ray showed a slight air bronchogram. Despite a further slight rise in respiratory rate and some auscultatory grunts, the baby remained pink in air and gradually improved. The baby also required treatment for hypoglycaemia, hypothermia and mild jaundice.

In summary, this was the child of a diabetic mother that suffered fetal distress in labour and was delivered by Caesarean section. It subsequently developed definite mild RDS (as confirmed by consultant paediatric opinion).

despite a mature L/S ratio of 2.5 and an NBS test of 22% orange staining cells both estimated 7 days before birth.

DISCUSSION

The most important investigations in planning an elective delivery are those which measure fetal physiological maturity. Tests which measure fetal size or body mass are less useful.

The L/S ratio measures the maturity of the fetal lungs and this is the limiting factor in the survival of the premature baby. We compared the L/S ratio to the NBS test and found a significant correlation. Quinlivan et al (15) found that creatinine and lipid containing cells (stained with oil red O) were more reliable in predicting fetal maturity than the L/S ratio. Kalbac et al (11) found the L/S ratio to be the best, creatinine next best and the NBS test the worst. However, both these investigations compared the laboratory tests to gestational age computed from the menstrual history rather than assessing the status of the baby produced.

It is useful to have more than one test available for the assessment of fetal maturity as technical factors may interfere with the laboratory analysis. Blood or meconium was present in 13% of the original samples of amniotic fluid and this is known to invalidate the L/S ratio. In such cases, reliable

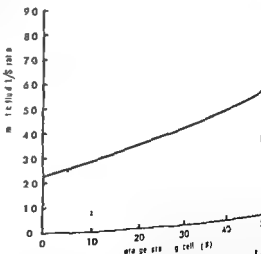


Fig 1 Graph of L/S ratio vs Nile blue sulphate test. L/S ratios obtained for 75 amniotic fluid specimens are plotted against the percentage of cells staining orange with Nile blue sulphate. All results with more than 50% orange staining cells have been graphed as 50% (0.001). The line of regression of Y upon X has been plotted.

would have to be placed on the NBS test which is unaffected by such factors. The counting of orange staining cells in the NBS test can however be influenced by clumping of the cells.

The outstanding situation in which rogue results seem to occur is in some cases of maternal diabetes mellitus. There was one such case in our series which developed RDS despite a mature L/S ratio. Previous investigators have found similar cases (3, 5, 6, 8, 12, 13, 14, 17) and it has been suggested by Whitfield et al. (17) that the L/S ratio might fall from a mature value in the interval between amniocentesis and delivery of the baby. With the exception of this one case RDS was successfully avoided in 72 induced deliveries. The L/S ratio and the NBS test were shown to correlate significantly with each other and to assist in planning the date of delivery.

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SERUM HCG AND HPL IN TWIN PREGNANCIES

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et Serum HCG and HPL levels were determined samples derived from 39 twin and one triplet nities. Compared with singleton gestations these were respectively 2.5 and 1.5 times higher through tation. Analysis of these data in terms of predictive cy for the existence of a twin indicates that neither serve for screening purposes though simultane termination of HCG and HPL may provide a fair f suspicion.

availability of radio immunological methods measurement of placental protein hormones ated interesting applications in the field of obstetrics. The quantitative assay of serum currently used for the early diagnosis of and extra uterine pregnancies for pre the outcome of threatened abortion for the is and follow up of patients with trophoblas eases and for monitoring cytotoxic drug used for treating these diseases. Measure serum levels of HPL is used for the assess placental function during the last trimester

se there is a correlation between placental and the serum concentration of placental e higher levels of both HCG and HPL be expected in multiple gestation. They have been reported by a number of investigators mainly in a limited number of cases. To question of the degree in which HCG PL assay might contribute to the early detec f multiple pregnancy serial determinations be performed in a sufficient number of pa

PATIENTS AND METHODS

HCG levels were determined by radio-im ssay using the dioxane precipitation method (5) ults being expressed in terms of the 2nd Interna

tional Standard HCG (MRC London England). For the determination of HPL a double antibody radio imunoassay technique was used (HCS Sciavo Sorin kit). All assays were performed in duplicate and serum sam ples were appropriately diluted with phosphate buf fered saline containing 1% bovine serum albumin to permit reading from the straight portion of the dose re sponse curve. For both hormone assays the mean inter assay coefficient of variation was 9%.

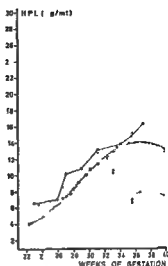
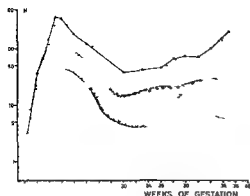
Because the HCG and HPL levels tend to have a log normal distribution throughout gestation statistical analysis of the data was performed accordingly. For HCG the geometric mean (± 1 S D) of normal singleton preg nancies was calculated from 1540 determinations in 830 women with known LMP. For HPL the calculations were based on 632 blood samples taken in 362 pregnancies. In 39 twin pregnancies and in one triplet gestation the serum levels of HCG and HPL were determined on at least two occasions. Preterm delivery occurred in 9 of these pa tients. Because the hormone levels characterizing the lat ter group were not divergent from those observed in the pregnancies carried until term (≥ 38 menstrual weeks) the twin data were pooled.

RESULTS

The individual levels of serum HCG and HPL are shown in Figs 1 and 2. The mean values calculated for two week intervals from the 25th week of gesta tion onwards were significantly higher in twin preg nancies. At the 36th week of gestation the mean (± 1 S E M) values of HCG and HPL amounted respectively to 39.1 ± 6.4 IU/ml and 14.7 ± 1.2 μ g/ml as against 14.5 ± 3.1 IU/ml and 10.7 ± 0.5 μ g/ml in singleton pregnancies.

Among 29 same sex twin sets (16 female and 13 male) 9 proved to be monozygotic. No in the mean serum levels of either or H according to zygosity was observed.

In contrast with singleton gestation the maternal HCG level w be higher for female conceptuses.



Figs 1 and 2 Serum HCG and HPL levels determined in 39 twin pregnancies. Shaded areas represent the geometric mean ± 1 SD of the normal singleton pregnancies. The solid line connecting the triangles indicates the HCG and HPL levels in a normal triplet gestation. The asterisks represent four pregnancies with intrauterine growth retardation.

The mean birth weight of 30 twin sets delivered after the 35th week of gestation amounted to 5359 ± 883 g (± 1 SD). As for the singleton pregnancies here too a significant correlation was found between the HPL level and the combined birth weight ($r=0.39$, $p<0.05$).

In the 9 pregnancies in which serum HCG could be assayed during the first trimester at least one value lay above the mean ± 1 SD. In 5 out of 9 cases the HCG levels exceeded the mean $+2$ SD. During the second and third trimesters of gestation at least one HCG or HPL level exceeded the normal range (represented here by mean ± 1 SD) in 28 (72%) and 29 (74%) of the 39 twin pregnancies respectively. However the HPL levels were found to lie consistently outside the normal range in only about half of the cases in which at least three determinations were performed at different times during pregnancy. When the data from both the HCG and the HPL assays are combined an elevated level of one or both of these placental hormones was found in 95% of the samplings. This might be explained by the lack of correlation between the HCG and HPL levels. In 3 out of the 4 pregnancies in which intra uterine growth retardation occurred (birth weight more than 2 SD below the mean) the value of serum HPL was abnormally low (Fig. 2).

DISCUSSION

It is well established that hormones whose pattern is directly related to placental and fetal growth tend

to attain higher concentrations in twin than in singleton pregnancies. Probably the first to observe this trend were Jones et al. (4) who reported elevated HCG levels in twin pregnancies in 1944. Later comparable observations were made for pregnancy related proteins and hormones. Wald et al. (6) found that in twin pregnancy maternal α fetoprotein levels were twice as high as for singletons which led these authors to warn that multiple pregnancy should be differentiated from those cases where the fetus had an open neural tube defect a condition which is likewise associated with elevated levels of α fetoprotein. In a study of the urinary oestrol excretion in 50 twin pregnancies Duff & Brown (2) reported the mean oestrol levels to be 1.7 times higher than in singleton pregnancies. From their data it appears that the assessment of fetal placental function on the basis of maternal urinary oestrol is only reliable when both fetuses are at risk. HPL levels were measured in 25 twin pregnancies by Ylikorkala (7) who found that the average concentration in the last month of pregnancy was 1.5 times higher than in singleton pregnancies and in 10 cases individual HPL values exceeded the 97.5 percentile. Abnormally low HPL values were observed in only one of the two cases with severe fetal placental dysfunction.

Recently Gennser (3) advocated use of the HPL assay as a screening test for multiple pregnancy. We too found that multiple pregnancy is associated with higher serum levels of HPL and HCG but in our opinion the fact that the diagnosis of twin gestation

cated by feto-placental dysfunction can easily be missed if one relies on the HPL assay. A constant major drawback of the method. Even though simultaneous determination of serum HCG and HPL may provide a better index for the existence of a twin pregnancy, we feel that the levels of these hormones are too inconsistent throughout pregnancy to satisfy the criteria for a useful screening procedure. Nevertheless, the findings indicate that when confronted with elevated levels of HCG in the first trimester of pregnancy as well as levels of HCG and/or HPL exceeding by 1 S.D. the levels of singleton pregnancies in the middle and third trimesters, the probability of a twin pregnancy should be kept in mind. Finally, HPL levels lying within 1 S.D. of the mean strongly suggest the existence of placental dysfunction.

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THE CERVICAL BALLOON METHOD FOR INDUCTION OF LABOR

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The cervical balloon (Embrey & Mollison) was used to induce labor in a study group of 87 patients. The control group included 97 patients where induction of labor was performed using oxytocin drip. In the study group oxytocin drip was added in 34 patients. The cases study and control groups were classified as favorable or unfavorable cervix. The patients classified as unfavorable cervix showed a shorter mean on-delivery interval in the study group and a shorter mean duration of oxytocin-drip in the cases of the study group where it was needed. It was concluded that the cervical balloon is a convenient method for the induction of labor for its effectivity, simplicity and innocuity in the unfavorable cervix cases.

Induction of labor is an obstetrical procedure having a questionable outcome and attempts have been made to introduce different methods in obstetrical practice. In spite of the trend in recent years to try various drugs (1, 2, 3, 4) or new approaches (5, 6), many obstetricians were attracted by the simplicity of the method using the cervical balloon described in the study by Embrey & Mollison (7). The purpose of this study is to report our experience of using this method in a group of patients.

MATERIAL AND METHODS

Initially two groups of pregnant women were selected where induction of labor had to be performed for different reasons. A randomization was made by introducing the women alternately in both study and control groups. Cases with ruptured membranes were excluded from both groups. The study group (Table I) included 87 selected patients who were distributed in two subgroups: the first 53 cases where the cervical balloon was introduced and where all the patients started labor and delivered without any oxytocic drug; the second subgroup 34 cases where the cervical balloon was introduced but where the patients needed oxytocin-drip for delivery. Usually the cases of this second subgroup were examined 24 hours after the catheter was introduced.

They were found not to be in labor although the cervix was ripe; the catheter had been expelled into the vagina and oxytocin-drip was started.

A 26 gauge Foley catheter was used and the 50 ml balloon was filled with 40 ml saline. After exposure of the cervix with a speculum, cleansing of the vagina and cervix, the catheter was introduced into the cervical canal until its balloon was just beyond the internal os and the balloon was then filled. No antibiotics were given preventatively. The control group included 97 unselected patients where a medical induction of labor with oxytocin drip was performed.

In all the cases oxytocin-drip was performed according to the following rules: primipara five units of oxytocin in

Table I Number of cases of the study group and control group

Method	No of cases	Total
<i>Study group</i>		
Cervical balloon	87	87
Cervical balloon+oxytocin-drip	34	
<i>Control group</i>		
Oxytocin-drip	97	97

Table II Pregnancy age at the time of induction

Pregnancy (weeks)	Cervical balloon	Cervical balloon+oxytocin drip	Study group (total)	Control group (oxytocin drip)
39	4	—	4	4
40	1	2	3	3
41	13	5	18	3
42	23	18	41	41
43	12	9	21	46
Total	53	34	87	97

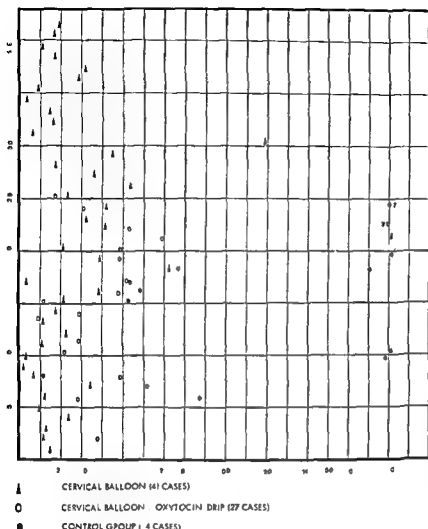


Fig 1 Comparative scattergram of induction-delivery interval (unfavorable cervix)

ml 5% glucose solution to undipara three units, multipara two units, quartipara and more one unit. The infusion was administered intravenously at a rate of 8–70 drops per minute. The women's ages which ranged from 19 to 40 were nearly homogeneous as also was the parity from primipara to quartipara and more. Pregnancy age at

the time of induction is seen in Table II. The indications for induction of labor included prolonged pregnancy at those cases ending the 40th week of pregnancy (mean gestational age) and were under observation in eliciting postmaturity.

In cases of pre-eclampsia the induction was performed in those where there were no signs of improvement after hospitalization and treatment. Other indications were diabetes, missed labor, uncoordinated contractions, placental insufficiency and Rh incompatibility. All the cases were classified either as favorable cervix or as unfavorable cervix (Table III) according to the definition of Embrey & Mollison (7). Cases with intermediate cervix were not included in this paper. All the cases were head presentation except three with breech presentation: one in the study group and two in the control group. The weights of the newborns in both the study group and the control group ranged from 2500 to 4500 g.

Induction-delivery interval: from the beginning of the induction till the delivery.

Oxytocin-drip time: the time that the patient is kept with the infusion-drip running.

Table III State of the cervix at the time of induction

Cervix	Cervical balloon	Cervical balloon + oxytocin drip	Group study (total)	Control group (oxytocin drip)
Favorable (ripe)	12	7	19	73
Unfavorable (unripe)	41	27	68	74
Total	53	34	87	97

Table IV Induction-delivery interval and oxytocin-drip time in the study group compared with the control group

	Cervical balloon Mean interval induct-del	Cervical balloon+oxytocin-drip		Control group (oxytocin-drip)	
		Mean inter induct-del	Mean time (oxytocin-drip)	Mean inter induct-del	Mean time (oxytocin-drip)
Unfavorable	12 cases 27 45 h	7 cases 25 14 h	3 32 h ± 0.52	73 cases 29 31 h	8 33 h ± 0.58
Unfavorable	121 00-4 10	49 55-7 10	5 0-1 00	407 40-2 15	35 50-2 15
Favorable	41 cases 28 39 h	27 cases 39 31 h	7 44 h ± 1.30	24 cases 86 32 h	15 15 h ± 1.33
Favorable	± 5.42	± 3.41		± 20.76	± 1.33
Favorable	202 35-2 70	76 15-7 00	27 00-0 50	407 20-4 30	33 30-4 30

COMMENTS

Table IV shows the results of our investigation. There was no substantial difference in mean induction-delivery interval between the cases in the unfavorable cervix groups. Only the possibly significant difference in the oxytocin-drip mean between the groups with favorable cervix is worth noting.

In the unfavorable cervix category we see that there is a clear difference between the induction-delivery interval of the study and control groups, as well as a marked difference when comparing the oxytocin drip times (Figs 1-4). The latter is explained by the fact that oxytocin drip, as we know, has more influence in a more decisive way when the ripeness of the cervix is completed. By introducing the cervical balloon, our cases achieved cervical ripeness in both of the study subgroups. We have no logical explanation why in one subgroup it developed promptly and in the other subgroup oxytocin drip was required.

In one case during an attempt to introduce the cervical balloon, slight bleeding started, placenta previa was presumed and the procedure was abandoned. The potential complications of the balloon method are accidental rupture of the membranes, displacement of the presenting part, and clasp of the umbilical cord. None of these was observed in our series. We had no cases of infection, although three cases of puerperal pyrexia were observed in the study group and two cases in the control group. No antibiotic therapy was instituted either in the study group or in the control group. In our experience we have seen that by means of

the cervical balloon we invariably obtain cervical ripeness and a number of cases went to delivery without any other measure. Even when oxytocin drip is also required, the time to reach the delivery

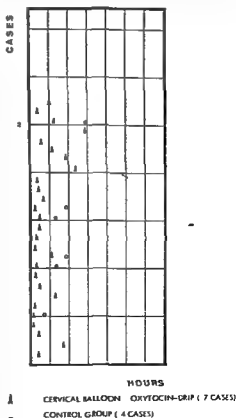


Fig. 2 Comparative scattergram of oxytocin-drip time (unfavorable cervix)

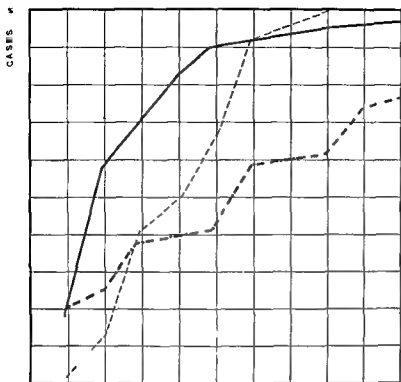


Fig 3 Cumulative curves of induction-delivery interval (unfavorable cervix)

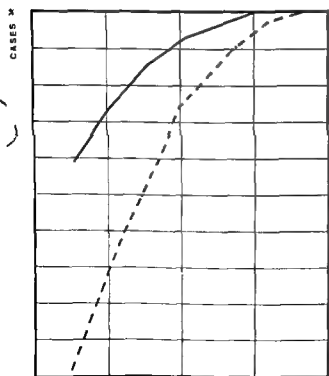
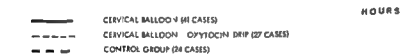


Fig 4 Cumulative curves of oxytocin-drip induction (unfavorable cervix)



er than the time needed with oxytocin drip
ered without previous cervical balloon
her point to underline is that the procedure can
nued in any time and another approach
assumed

rally perhaps we have the feeling that we
beyond the era of the cervical bal
but the experience reported here and by
& Mollison (7) themselves shows that this
proved to be an efficient and innocuous
and merits that it would be wise to take it into

Further investigations about the use of
prostaglandins following insertion of a cervical bal
are in progress

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EVALUATION OF A FETO PELVIC SCORING SYSTEM IN THE MANAGEMENT OF BREECH PRESENTATIONS

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Abstract A feto-pelvic scoring system comprising maternal pelvimetric data, estimated fetal weight, type of breech presentation and previous obstetric history was used in selecting patients for cesarean section or vaginal delivery. A maximum score of 20 points was possible. Twelve points or less indicated cesarean section. During 1973-1975 224 singleton breech deliveries were evaluated. In 29.5% cesarean section was performed and in 83% of these it could be planned in advance. In 70.5% of cases patients were allowed to deliver vaginally under continuous electronic monitoring of the fetal heart rate. There was one intrapartum death and only one early neonatal death of a small premature child. In two cases intrauterine death had occurred already in the antepartum period. The uncorrected perinatal mortality was 17.9 per 1000 but not significantly different from the uncorrected perinatal mortality of 8.0 per 1000 for all patients delivered at the Danderyd's Hospital during the period 1972-1975 (12832 births). The corrected mortality resulting from breech presentation was 8.9 per 1000. The infants exhibited similar and excellent 5 min Apgar scores whether delivered vaginally or by cesarean section or matched with a randomized control series of 1000 cephalic presentations.

The relative contribution of breech presentation to perinatal morbidity and mortality is increasing as deaths from other causes decline.

From an international point of view perinatal mortality in Sweden is low, making the relative contribution of breech presentation to perinatal death rate even more crucial (8). The need for improved methods in the management of breech presentations is therefore urgent.

In 1974 the present author (20) described a scoring system based on maternal pelvimetric data, estimated fetal weight, type of breech presentation and previous obstetric history. The advantages of this scoring system was proved to be suitable for evaluation of whether the patient before the onset of labour was a candidate for planned cesarean section or vaginal delivery. In a preliminary prospective series using this scoring system one third of the

patients underwent planned cesarean section while the remaining two thirds were allowed to deliver vaginally. In this series the infants exhibited similar and excellent results at the 5 min Apgar score (1) whether delivered vaginally or by cesarean section and as matched with a double control series of cephalic (occipito-anterior) presentations. The present paper is an extension of the above mentioned prospective study.

MATERIAL

During the period 1973-1975 224 singleton breech presentations at the Danderyd's Hospital delivered vaginally or by cesarean section were evaluated according to the previously described breech index (20).

METHOD OF MANAGEMENT

X-ray pelvimetry (3, 4, 5) was performed on all patients independent of parity when fetal weight was estimated to be well above 2000 g. Exceptions to this rule were the previously uncomplicated delivery of a large fetus as a breech presentation and obvious indications for cesarean section other than feto-pelvic disproportion.

Scoring of a breech. The main points considered were: 1) pelvimetric data, 2) estimated fetal weight, 3) type of breech presentation and 4) previous obstetric history.

Each variable could have a score from 0-2 points and the total maximum score was 20 points. The score should exceed 12 for safe vaginal delivery. Single measurements of the pelvic inlet or outlet could have a score of 1. In breech presentation sagittal and transverse pelvic inlet scores are exchangeable. Zero scores excluded vaginal delivery (20).

Fetal weight was estimated by a combination of *symphysis fundus tape measurement* (comparison with a SF/fetal weight curve) and *palpation* (20). According to the above mentioned method the mean deviation from true mean infant weight was minus 2 g. In 96 randomized estimates (true fetal weight range 1700-4600 g) the standard deviation was ± 254 g. All estimations were performed by midwives.

Cesarean section was indicated when

1. One or more pelvic measurements scored zero or total score was ≤ 12 .

Table I *Feto pelvic scoring system in the management of breech presentation (20)*

Parameter	Pelvimetry and fetal weight	0	1	2	Score
Sagittal inlet		<11.5	11.5-17	>17	
Transverse inlet		<12.5	12.5-13	>13	
Interspinal dist		<10	10-10.5	>10.5	
Intertubar dist		<10	10-11	>11	
Sagittal outlet		<10.5	10.5-11	>11	
Sum of outlet		<32.5	32.5-33.5	>33.5	
Estimated fetal weight g		<1 500 >4 000	1 500-2 000 3 500-4 000	2 000-3 500	
Presentation		Double footling None Uncompl breech <2 kg Uncompl headpresent <3 kg Complicated delivery	Complete breech Single footling Uncompl breech 2-3 kg Uncompl headpresent >3 kg	Frank breech Uncompl breech >3 kg	
Previous vaginal deliveries		Unripe cervix and rigid pelvic floor	Unripe cervix or rigid pelvic floor	Ripe cervix and relaxed pelvic floor	
Soft birth canal					

- 2 One or more pelvic measurements scored 1 and fetal weight was estimated to be above 3 500 g
- 3 Fetal weight was estimated to be more than 4 000 grams or less than about 1 500 g (Crush injury) (16)
- 4 Double footling was present (high incidence of prolapsed cord) (17)

Supervision of labour

- 1 Continuous FHR monitoring was mandatory. The midwives were not allowed to leave the patient.
- 2 Second stage of labour was *shortened* by intravenous oxytocin infusion because of the risk of cord compression (18) and fetal acidosis (12).
- 3 *Pudendal block* (or epidural) was used in all vaginal deliveries to relax the pelvic floor. Perineotomy was mandatory in all nulliparae and elsewhere when needed.
- 4 Attendance of obstetrical, paediatrician and anaesthetist at the end of the second stage of labour was obligatory.
- 5 If there were difficulties in delivering the *aftercoming head* an assistant introduced two fingers in the maternal rectum above the fetal head. Pressure in a caudal direction aided the delivery of the head.

In some additional cases the pelvis was too small for a breech but appropriate for a cephalic presentation. Gentle external version was performed after the 35th week of gestation. No anaesthesia was used but the uterus was relaxed by a terbutaline infusion. Vaginal deliveries of the cephalic presentations occurred without complications.

RESULTS

In 42 nulliparae and 24 multiparae caesarean sections were performed with a caesarean section rate of 29.5% (Table II). In 83% of these planned caesarean sections could be performed. In two thirds of the cases the indication was fetopelvic disproportion

according to the breech index. In 17% and for various reasons as indicated in Table II emergency caesarean section had to be performed. There were no perinatal deaths and no infant had an Apgar score of 1-3 at 5 minutes (Table III). In 10.5% assisted vaginal delivery occurred. There were 68 nulliparae and 90 multiparae. There was one intra-partum death resulting from failure to use fetal heart rate monitoring. One early neonatal death occurred of a premature baby weighing less than 1 500 grams. In two cases fetal death had occurred before the onset of labour (Table III). The uncorrected perinatal mortality for 224 breech presentations was thus 17.9 per 1 000 which may be compared with an uncorrected perinatal mortality of 8.0 per 1 000 for all patients delivered at the Danderyd's Hospital during the period 1972 to 1975 (12 832 births). If correction is made for the antepartum deaths in the breech series the corrected perinatal mortality was 8.9 per 1 000. No infant delivered vaginally had an Apgar score of 1-3 at 5 minutes. As is shown in Table III vaginal delivery of breech presentations compared favourably with caesarean sections and the results were only slightly but not significantly different from a randomized sample of 1 000 cephalic presentations.

DISCUSSION

Comparisons have been made on large clinical series between cephalic and breech presentations (6, 9, 17) and the increased complication rate in the

Table II Indications for cesarean section (29.5%)

Breech presentation (42 nulliparae 24 multiparae)

Indication	Number of cases	Distribution of planned and emergency cesarean sections
Ind CS		
Narrow pelvis	41	
Fetal weight >4000 g	3	
Large pelvis		
Mal-ornate uterus	4	
Plac. praevia	2	
Preeclampsia	2	
F.U. growth retard	1	
Maternal congen. muscle-dystrophia + sterilization	1	55 (83%)
Previous cerebral palsy	1	
Emergency CS		
Narrow pelvis		
Prolapsed cord	4	
Prim. ut. inertia	1	
Imminent uterine rupture	1	
Double foetus	1	
No X-ray		
Abruptio plac.	1	
Prematurity	3	11 (17%)

breech presentation has been stressed. With the exception of a few studies (13, 15, 17) guidance is vague for the management of breech presentations. In order to diminish asphyxia and birth trauma in breech presentation the Zatuchni-Andros breech scoring index (21) was tested at the Danderyd's Hospital. However, we were unable to verify the prognostic value of this index (20). The incidence of Apgar score less than 8 at one minute was lower (33%) when the breech score was poor (0-3) than

when it was high (breech score 4-8, Apgar score less than 8 at one minute in 50%). This type of breech index also has the disadvantage of not allowing planned cesarean sections to be performed.

For more than a decade excellent pelvimetric X-ray methods have been available for obstetric use in Sweden (3, 4, 5). These methods were originally designed for occipito-anterior vertex presentations. In spite of this the same pelvic outlet borderline values have been used by most Swedish obstetricians for breech deliveries.

It has to be realized that unmoulded heads after breech deliveries have much larger skull diameters than moulded heads of similar sized infants after vertex deliveries (20) which is one of the reasons why the pelvis must be roomier for breech than for cephalic presentations. A second reason is that the shoulder width is of the same order of magnitude as the suboccipito-bregmatic diameter even if the shoulders are firmly compressed. A third reason is that the cross-sectional diameter (fetal head plus arms) when both arms are extended over the fetal head is larger than the fronto-occipital diameter (20).

In 1971 and 1972 at the Danderyd's Hospital the risk of having an Apgar score less than 8 at one minute was nine times greater in breech presentation than for all newborn infants during the period (20). In the present paper the one minute Apgar score was omitted because it is not significantly related to cerebral palsy or mental retardation (2). For this reason the five minute Apgar score was chosen which appears to be related to neuro-pediatric handicaps. Although no less than two thirds of cerebral palsies and mentally retarded cases originate from intrauterine growth retardation the contribution of traumatic breech delivery to neuro-pediatric handicaps can not be neglected any

Table III Apgar score in singleton breech deliveries at 5 min
Breech n=224

Presentation method of delivery	5 min Apgar score				Number of cases
	0	1-3	4-7	8-10	
Breech vaginal 70.5%	1.9%	0.0%	2.5%	95.6%	Nulliparae n=68 Multiparae n=90
Breech Cesarean section 29.5%	0.0%	0.0%	4.5%	95.5%	Nulliparae n=47 Multiparae n=24
Cephalic vaginal randomized sample 1974 n=1 000	0.4%	0.1%	1.5%	98.0%	Nulliparae n=397 Multiparae n=603

2 amniotomies before admission to hospital

longer. For this reason cesarean section for all breech presentations has been recommended (10).

However the present investigation indicates that cesarean section rate can be reduced to about 30% allowing the remaining 70% to go into spontaneous labour with minimal risk. The single tragic intrapartum death could probably have been avoided had our recommendations for continuous electronic fetal heart rate monitoring been followed.

No surviving infant had an Apgar score of 1-3 at five minutes. The vaginal breech deliveries were as safe as those where cesarean sections were performed and the whole group of breech presentations performed nearly as well as a big randomized sample of cephalic presentations.

In a recent Swedish publication (19) the main points of the present author's fetopelvic scoring system (20) have been used prospectively and the results of the present paper have been confirmed.

From a practical point of view estimation of fetal weight in the present investigation by a combination of symphysis fundus tape measurement and abdominal palpation proved to be satisfactory and better than palpation alone which has been strongly criticized (14).

Undoubtedly more precise weight estimations can be made from ultrasonic measurements of fetal abdominal circumference (7-11). However in the present case series it has not been possible to utilize sonography because of lack of funds for such

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IMMEDIATE POST PARTUM INSERTION OF THE ANTIGON

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Abstract The results of intrauterine contraception using the Antigon inserted into 364 women immediately post partum are submitted. The patients were followed for 24 months total number of woman months 5225. The follow up was 96.7% after 3 months, 97.9% after 12 months and 89.7% after 24 months. Four types of Antigon were used having surface areas from 785 to 2740 mm². Type I and the wing model (type IV) which have the smallest surface area proved best suited. The continuation rates for types I and IV after 12 and 24 months were 77.4-59.2 and 76.4-54.5 respectively. These rates are on a level with those for Antigon inserted 6-8 weeks post partum. It is notable that the expulsion rates (12.8 and 12.7 after 12 months) were no higher than those for Antigons inserted 6-8 weeks post partum. The incidence of puerperal complications was not increased and no perforations occurred.

During and immediately after a pregnancy a woman is often well motivated for contraceptive guidance, but this motivation sometimes falls off soon after delivery so that she does not keep the appointment for follow up 6-12 weeks post partum (1, 2). It would be a help to these women to have an intrauterine device (IUD) inserted before they leave hospital after delivery. Owing to a fear that the insertion of an IUD immediately post partum might increase the risk of infection, bleeding and perforation it has previously been recommended to wait until 10-12 weeks post partum (3). However, a number of reports have shown that this fear is unfounded (4-10) and in a recent publication it was concluded by Rosenfield et al. (11) that insertion of an IUD soon after delivery is safe and demographically the potentially most effective form of contraception. The IUD most often used has been Lipps loop D generally inserted 2-4 days post partum.

In the Department of Obstetrics and Gynecology section YB Rigshospitalet University of Copen-

hagen the Antigon has been inserted in a number of cases since 1967 just after delivery of the placenta. The results are submitted below.

MATERIAL AND METHOD

During the period 1967-1972 patients delivered in the Department and who wished intrauterine contraception were offered insertion of an Antigon immediately post partum. 364 chose insertion at this time whereas 914 preferred waiting until 6-8 weeks after delivery. Four different types of Antigon were used (Fig. 1). The original model (type I) first described by Osler & Lebech (12) is kite shaped made of polyethylene measures 30x23 mm and has a surface area of 785 mm². In the bag model (type III) the original Antigon is placed in a plastic bag. Its surface area is 2740 mm². The wing model (type IV) is furnished on the inside with plastic wings connected by a cross. Its surface area is 1057 mm². In Antigon F studied by Fuchs et al. (13) the frame is filled with a thin plastic membrane with horizontal slits. Its surface area is 1725 mm². The Antigon has a built in magnet so that it can be checked by a galvanometer.

Immediately after delivery of the placenta and after it had been ascertained that the uterine cavity was empty the Antigon was inserted manually up towards the fundus when which was at the same time supported from the outside. Methyl ergometrine was administered intravenously (Methergin Sandoz Basle) and when the uterus had contracted the hand was cautiously withdrawn leaving the Antigon in the uterus. During the insertion a light nitrous oxide anaesthesia was occasionally given.

83 women had type I, 133 type III, 83 type IV and 67 Antigon F inserted. At discharge 5-7 days post partum gynecological examination was carried out supplemented by a galvanometer test to check whether the Antigon was in situ. Moreover follow up examinations were performed at 3, 12 and 24 months. No other form of contraception was used. Tables I and II give the distribution by age and parity. The insertion as well as the follow up examinations were in the hands of changing members of the obstetrical staff. The follow up rate was 96.7% after 3 months, 97.9% after 12 months and 89.7% after 24 months. Data were analysed by the life table method (14).



Fig. 1

RESULTS

Table III presents the cumulative event rates after 12 and 24 months for the 4 types of Antigon. The 364 patients were followed for a total of 5225 months. Reinsertion of the Antigon was rarely done, as is apparent from the continuation rates as compared with the total event rates.

Pregnancies Five women conceived during the first years. One with type I, none with type III and two each with type IV and Antigon F, making pregnancy rates of 1.4, 0, 3.1 and 3.8 respectively. The differences are not significant. Four conceived during the second year. Two with type I, one with type III, none with type IV, and one with Antigon F, making cumulative pregnancy rates of 5.7, 2.2,

Table I. Age distribution for 4 types of Antigon

Years	Type I	Type III	Type IV	Antigon F
<18	3 (3.7%)	2 (1.5%)	0	0
18-24	27 (33.3%)	67 (50.4%)	30 (36.1%)	13 (19.4%)
25-29	22 (27.2%)	33 (24.8%)	26 (31.3%)	24 (35.8%)
30-34	16 (19.8%)	18 (13.5%)	17 (20.5%)	23 (34.3%)
>35	13 (16.1%)	13 (9.8%)	10 (12.1%)	7 (10.5%)
Total	81	133	83	67

Table II Distribution by parity for 4 types of Antigon

Parity	Type I	Type III	Type IV	Antigon F
I	14 (17.3%)	44 (33.1%)	30 (36.1%)	19 (28.4%)
II	23 (28.4%)	41 (30.8%)	25 (30.1%)	24 (35.8%)
III	44 (54.3%)	48 (36.1%)	28 (33.7%)	24 (35.8%)
Total	81	133	83	67

51 and 6.6 respectively. The differences are not significant.

Expulsions: 73 Antigons were expelled during the first year, including 46 during the first month post partum, 15 during the second month, and 2 during the third month. From the 4th to the 12th month 10 Antigons were expelled. Of the expelled Antigons 48 were of type III, 10 each of types I and IV, and 5 of the Antigon F type. The corresponding expulsion rates after 12 months were 39.1, 12.8, 12.7, and 7.9. The expulsion rate for type III is significantly higher ($P < 0.05$) than for the other types, whereas no significant differences were found between types I, IV, and Antigon F. Only 5 were expelled during the second year—1 each of types I and IV, 3 of type III, and no Antigon F. There are no significant differences in the expulsion rates between the types during the second year.

Removals: 34 Antigons were removed because of bleeding/pain during the first year: 4 of type I, 11 of type III, 6 of type IV, and 13 of type Antigon F. The removal rates of 5.6, 14.0, 9.0, and 22.1. The removal rate for types I and IV is significantly lower ($P < 0.05$) than for Antigon F, but not for type III.

During the second year 21 Antigons were removed: 4 of type I, 9 of type III, 5 of type IV, and 3 of type Antigon F. This makes the cumulative removal rates after 24 months 12.8, 29.9, 17.7, and 28.8. The rate for type I is significantly lower ($P < 0.05$) than for type III and Antigon F, but otherwise there were no significant differences between the types. Only a few removals were done because of other medical or personal reasons, but 22 because of planned pregnancy, which gives cumulative removal rates at 24 months of 5.7, 15.9, 11.4, and 13.8 for types I, III, IV, and Antigon F.

Continuation rates at 12 and 24 months were 77.4 and 59.2 for type I, 41.5 and 13.1 for type III, 76.4 and 54.5 for type IV, and 64.3 and 38.1 for Antigon F. When deducting removal because of planned pregnancy, the acceptability rates after 24 months are 9.2, 29.0, 65.9, and 51.9 respectively.

Nine patients (2.5%) had either low grade temperature/tenderness of the uterus and/or foul smelling discharge during the puerperium. This incidence is no higher than the ordinary incidence of puerperal infection. Five (1.4%) had increased vaginal bleeding. There were no instances of major

Table III Net cumulative event rates (\pm S.E.) per 100 women at 12 and 24 months for the 4 types of Antigon inserted immediately post partum

Months of use	Type I		Type III		Type IV		Antigon F	
	12	24	12	24	12	24	12	24
Pregnancy	1.4 \pm 1.4	5.2 \pm 3.0	0	2.2 \pm 2.2	3.1 \pm 2.2	3.1 \pm 2.2	3.8 \pm 2.6	6.6 \pm 3.8
Expulsion	12.8 \pm 3.8	14.5 \pm 4.1	39.1 \pm 4.1	43.1 \pm 4.7	12.7 \pm 3.8	14.5 \pm 4.1	7.9 \pm 3.4	7.9 \pm 3.4
Removals								
Bleeding/pain	5.6 \pm 7.7	12.8 \pm 4.3	14.0 \pm 3.9	29.9 \pm 5.8	9.0 \pm 3.5	17.7 \pm 4.9	22.1 \pm 5.4	28.8 \pm 6.7
Other medical	2.9 \pm 7.0	4.8 \pm 7.7	4.0 \pm 2.3	6.2 \pm 3.1	0	0	0	2.9 \pm 2.9
Planning pregn	0	5.7 \pm 3.2	1.4 \pm 1.4	15.9 \pm 5.7	0	11.4 \pm 4.4	0	13.8 \pm 5.6
Personal reason	0	0	4.0 \pm 2.3	4.0 \pm 2.3	0	0	1.9 \pm 1.9	1.9 \pm 1.9
Total event rate	77.7	43.0	67.5	101.3	74.8	46.7	35.7	61.9
Continuation rate	77.4	59.2	41.5	13.1	76.4	54.5	64.3	38.1
Woman months of use	877	1437	876	1404	761	1357	644	1077
Number of first insertions	81		133		83		67	

Table IV Net cumulative event rates (\pm SE) per 100 women at 12 and 24 months for the 4 types of Antigon

Months of use	Type I		Type III		Type IV		Antigon F	
	12	24	12	24	12	24	12	24
Pregnancy	3.8 \pm 1.3	9.6 \pm 2.1	2.5 \pm 1.4	2.5 \pm 1.4	5.0 \pm 1.6	5.7 \pm 1.8	3.1 \pm 1.2	3.1 \pm 1.1
Expulsion	10.3 \pm 1.9	11.4 \pm 2.1	24.8 \pm 3.7	27.6 \pm 3.9	11.3 \pm 2.3	11.9 \pm 2.4	8.9 \pm 2.0	9.6 \pm 2.1
Removals								
Bleeding/pain	8.3 \pm 1.8	12.2 \pm 2.2	11.1 \pm 2.8	19.8 \pm 3.9	15.7 \pm 2.6	24.9 \pm 3.3	14.4 \pm 2.4	26.1 \pm 3.3
Other medical	1.7 \pm 0.9	2.3 \pm 1.0	1.7 \pm 1.2	2.9 \pm 1.7	0.6 \pm 0.6	0.6 \pm 0.6	1.0 \pm 0.7	2.6 \pm 1.3
Planning pregn	3.0 \pm 1.1	7.7 \pm 1.9	4.9 \pm 2.0	17.5 \pm 3.9	0.6 \pm 0.6	7.9 \pm 2.3	3.6 \pm 1.3	12.7 \pm 1.7
Personal reason	1.7 \pm 0.9	2.3 \pm 1.0	0.8 \pm 0.8	2.1 \pm 1.5	1.7 \pm 1.0	2.4 \pm 1.1	1.0 \pm 0.7	4.1 \pm 1.7
Total event rate	23.8	45.5	45.8	72.4	34.9	53.4	32.0	57.7
Continuation rate	73.9	59.6	59.7	38.8	66.0	47.6	68.3	47.5
Woman months of use	2 773	4 712	1 433	2 359	2 111	3 669	2 373	3 865
Number of first insertions	278		161		277		248	

pelvic infection or bleeding disturbances and no perforations occurred

DISCUSSION

In accordance with a number of other investigations (4-10) the present study showed that insertion of an IUD immediately or soon post partum does not involve an increased risk of puerperal infection. We found increased bleeding in 5 patients (1.4%) during the puerperium. Previous authors have reported between 2% and 24% (11). Such a wide variation is presumably due to a difficulty in assessing the extent of the bleeding. Hingorani et al (15) found no difference in hemoglobin level between two groups of women with and without insertion of an IUD post partum. Davis (3) has recommended waiting until 10-12 weeks after delivery at which time the risk of perforation is said to be least. His conclusion is based upon the data of Ratnam et al (16). Their publication shows, however, that the risk of perforation is only 0.24% at insertion within 48 hours post partum against 1.8% 4-8 weeks later and 0.42% more than 8 weeks later. Moreover, the majority of the perforations were due to an incorrect technique of insertion (16). We did not observe perforations among the 364 patients who had Antigons inserted immediately after delivery of the placenta and Banharnsupawat et al (9) found no perforations among 7 172 women who had Lippes loop inserted in most cases 2-4 days post partum. Thus, there is nothing to indicate that the risk of perforation is increased by inserting an IUD immediately or soon post partum.

Of the four types of Antigon, I and IV had the highest continuation rates at 12 as well as at 24

months: 77.4-59.2 and 76.4-54.5 against 41.5-13 for type III and 64.3-38.1 for Antigon F. The bag model (type III) which has the largest surface area has a significantly higher ($P<0.05$) expulsion rate, (39.1 after 12 and 43.1 after 24 months) than other types and a very high removal rate after 24 months (29.9) which renders this model unsuitable as an IUD immediately post partum. The removal rate for Antigon F because of bleeding/pain at 12 and 24 months was 22.1 and 28.8 respectively or significantly higher ($P<0.05$) than for type I and significantly higher ($P<0.05$) than for type IV after 12 but not after 24 months. The cause of the differences in removal rates may be partly a difference in surface area (Antigon F has the largest surface area) and partly the fact that Antigon F was used during a later period than types I and IV. Doctor and patient attitude may have changed and this may have greatly influenced the removal rates (17). Despite marked differences in surface area (785 to 2740 mm²) there are no significant differences in the pregnancy rates between the four types of Antigon. This may be due partly to the small size of the series and partly to the fact that 3-6 months elapse before full fertility has been re-established after delivery (18). Therefore, any difference in the pregnancy rate will not be able to manifest itself at least during the first year.

The continuation rate for types I and IV Antigons is on a level with that for Lippes loop D inserted 2-4 days post partum (9-11) but the expulsion rate (19.0 and 23.4 at 12 months) as well as the removal rate (21.1 and 23.0) are higher for Lippes loop than for the Antigon. With Lippes loop, however, the reinsertion percentage has been very high (85 and

which greatly reduces discontinuation because of expulsion. After insertion of Burnberg's bow (7) and the safety filament bow (10) immediately post partum the expulsion rate has been at the same low level as in our study but the expulsion rate for Lippes' loop (11) is higher on insertion immediately post partum than 2-4 days later (11). The difference in the expulsion rates may be due partly to differences in the technique of insertion and partly to the Antigon bow and safety filament bow adapting better to a uterus during sub-involution.

The results of inserting an Antigon immediately post partum are on a level with those of insertion 6-8 weeks later. This is apparent from Table IV which shows event rates at 12 and 24 months for the four types of Antigon inserted during the same period (1967-1972). It is noteworthy that except for type III the expulsion rates are not higher for the Antigons inserted immediately post partum than for those inserted 6-8 weeks later. There are also no significant differences in pregnancy or removal rates. The continuation rates were a little higher for types I and IV and a little lower for Antigon F on insertion immediately post partum than 6-8 weeks later. The differences are not significant.

As insertion of an Antigon immediately post partum does not involve an increased risk of puerperal complications or perforations and as the results are fully up to those on insertion 6-8 weeks later insertion immediately post partum is preferable in general since this is the time at which the patient is most interested in contraception. In the present study there was no difference in the results between types I and IV Antigon but type IV is preferable as previous investigations (19, 20) have revealed far higher pregnancy rates for type I.

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IMMUNITY TO MODIFIED SEMINAL PLASMA ANTIGENS ASSOCIATED WITH SUBFERTILITY

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Abstract The technique of counterimmunoelectrophoresis (CIE) has been used to detect precipitin reactions between sera from subfertile and fertile patients and seminal plasma antigens. Freshly ejaculated seminal plasma lacked antigenicity detectable by CIE. Seminal plasmas obtained as part of routine semen analyses and therefore left in stand for varying periods showed activity. In a study of 60 spouse seminal plasmas which were collected in routine fashion and then incubated at 37°C antigenic activity appeared after varying periods of incubation. Activity was most commonly detected after 18 hours incubation usually lasting for 6 hours or more. A pool of 37 seminal plasmas (PSP) which was rendered antigenic by incubation reacted on CIE with 156 (83.7%) of 187 sera from subfertile patients and with 80 (63.0%) of 127 sera from fertile women. In three of the positive sera the immunoglobulin fraction was recovered and found to contain the components binding with PSP antigen. The PSP material was bacteriologically contaminated and on culture 7 bacterial types were isolated—*Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus subtilis*, non haemolytic and haemolytic diphtheroids, *Mucoid E. coli* and *Streptococcus faecalis*. Pooled cell free extracts (CFE) from these bacteria reacted with 154 (84.6%) of the subfertility patients and 68 (53.5%) of the comparison subjects. Use of a 3 well CIE procedure revealed the presence of some of these CFE bacterial antigens in PSP. A component distinct from the bacterial antigens was observed in PSP. This antigenic component is thought to represent seminal plasma antigen(s) resulting from bacterial action. Its appearance is induced by contact of seminal plasma with any of the 7 bacterial types but not by incubation of sterile seminal plasma alone or in combination with CFE or bacterial protease. A proportion of the bacterial antigen(s) was heat labile. Those that were thermostable were removed from PSP by absorption with chicken anti bacterial antisera. The absorbed PSP reacted with 58 (31.1%) of the subfertile subjects and 15 (11.8%) of the fertile women. The subfertile group was subdivided into primary and secondary subfertility and again according to whether conception occurred during the course of the study. A difference in CIE positivity was observed in the secondary but not in the primary subfertile women. Twenty two (61.1%) of the 36

secondary subfertile women who failed to conceive were CIE positive in contrast to 8 (70.7%) of the 29 women who became pregnant ($p < 0.005$).

The antigenic properties of human seminal plasma as demonstrated in experimental animals are well known (7-13). Heterologous antibodies raised against various components in seminal plasma are easily detectable by commonly used techniques such as gel precipitation and passive haemagglutination with tanned erythrocytes (2, 7-8).

There are very few publications on the existence of homologous antibodies to seminal plasma antigens so little is known of the immunity to seminal plasma in man. There have been reports of reaginic type reactions occurring in females immediately after sexual intercourse (6, 9, 10). However such cases are extremely rare. The antigen responsible for the anaphylactic phenomenon has been characterized as a low molecular weight non sperm coating seminal plasma protein. Immune reactions to other seminal plasma components have been investigated using the techniques of gel precipitation and passive haemagglutination (8). These procedures which work well with heterologous antisera have given negative or at best doubtful results when human sera were tested. The explanation for the results may be in part insensitivity of the techniques employed and in the low activity of the homologous sera.

Employing the technique of counterimmunoelectrophoresis (CIE) Chen et al demonstrated the presence of antibodies to seminal plasma in the sera of infertile women and prostitutes (2, 3, 4, 5). Carretti has also reported finding antibodies to seminal plasma components in the sera of w.c.

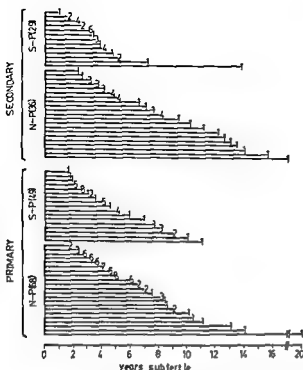


Fig 1 Composition of subfertility patients primary and secondary subdivided into those that remained non pregnant (N P) and those that subsequently became pregnant (S P). The number in each group is given in parenthesis. Each line represents a common period of subfertility shared by a number of patients (given in numerals at the end of the line)

unexplained infertility using the technique of reversed passive haemolysis of cells coated with seminal plasma (1).

In our recent studies we observed that serum precipitating activity of seminal plasma under CIE appeared on standing and storage but was not present in freshly collected semen. In this paper factors associated with the appearance of activity in seminal plasma are described. The frequencies of reactivity to modified seminal plasma antigens have been determined in 182 subfertile women and in 127 comparison subjects.

MATERIALS AND METHODS

Sera for the study were obtained from 182 patients who had no apparent organic cause for their inability to conceive and who attended the University Unit, Kangar Kerbau Hospital for Women, Singapore, during the period 1969–1976. These patients were thoroughly investigated by clinical examination, dilatation and curettage, tubal insufflation and where necessary, hysterosalpingogram and laparoscopic hydrotubation. Full semen analyses of

spouses of the patients were also performed. The group of 182 patients consisted of 117 who had not been pregnant previously and who despite regular coitus over a period of 2 years or longer failed to conceive (primary subfertility). The remaining 65 had previously conceived but had failed to conceive again after attempting for a year or more (secondary subfertility). The duration of their subfertility is shown in Fig 1. Their ages ranged from 20 to 38 years with a mean of 28.6 years at the time of first consultation.

Sera from the 127 fertile patients were also tested. The sera comprised 111 from pregnant women, 14 from women in the immediate postpartum period and 21 multipara with a parity equal or greater than 5.

Reactivity of freshly obtained seminal plasma. A fresh ejaculate from a healthy male was obtained. Seminal plasma was prepared immediately by centrifugation at 3000 r.p.m. for 15 min. The supernatant seminal plasma was tested for reactivity with sera from 30 subfertile women by CIE. These sera were previously found to be positive to pooled modified seminal plasma. The conditions employed for CIE were as described before (4) except that 0.85% agarose (same source) was used and electrophoresis was continued for 75 min.

Influence of incubation on spouse seminal plasmas. Semen from 60 spouses was collected by masturbation into clean containers. After standing at room temperature for about 6 hours for routine analysis, the semen samples were held at 4°C prior to seminal plasma recovery. Each seminal plasma specimen was separately incubated at 37°C and aliquots removed at 6-hourly intervals. These were tested by CIE for activity against the corresponding spouse serum and a standard positive subfertility serum (K228).

Preparation of pooled seminal plasma (PSP) and screening of study sera. Seminal plasma from 31 healthy donors (comprising 34 Chinese, 1 Malay and 2 Indians) were prepared after routine semen analysis had been performed. The specimens were separately incubated at 37°C and activity tested by CIE using 6-hourly interval aliquots and K228 serum. When a titre of activity of 1:1 or higher was reached, each specimen was stored at -20°C and eventually all were pooled. The sera in both subfertile and fertile study groups were tested on CIE in doubling dilutions against PSP, also in doubling dilutions. Five sera including K228 reacted strongly on CIE and were used as a positive sera panel.

Demonstration of immunoreactivity in the CIE reactions. Three of the more strongly CIE positive subfertility sera were selected and their globulin fractions separated out with a 12% concentration of sodium sulphate. The fractions were dialysed against 0.01 M phosphate buffered saline, pH 7.5 (PBS) and applied to 4% Diethylaminoethyl cellulose columns in order to obtain the immunoglobulin fraction. CIE was performed between PSP and this immunoglobulin fraction.

Bacteriological studies on seminal plasma. PSP was examined microscopically for the presence of bacteria. Cultures were made initially on blood agar and subsequently in nutrient broth.

Reactivity of study sera to bacterial cell free extracts (CFE). The bacteria from PSP were cultured in nutrient

broth provide cell free extracts which were then used to screen all the sera for antibacterial activity by CIE

Immunochemical relationship of antigens in PSP and CFE To investigate the immunochemical relation between the antigenic determinants in PSP and CFE a 3-well CIE procedure was developed involving 2 antigen wells one immediately above the other and a single antibody well 4 mm distant and punched opposite the mid point of a line joining the centres of the 2 antigen wells as illustrated in Fig 3 One hundred and fifty nine sera from infertile women and 49 sera from comparison subjects were tested

Characterization of antigens in seminal plasma Seminal plasmas were obtained from freshly ejaculated semen from 4 healthy donors pooled and filtered through a millipore membrane (Millex Filter Unit (0.22 µm pore size)) to exclude bacteria The pooled seminal plasmas (PS) were confirmed to be non-reactive when tested with the 5 panel sera and used as follows

- 1 an aliquot was kept sterile
- 2 equal volumes of millipore filtered CFE were mixed with equal volumes of PS and PBS (control)
- 3 equal volumes of pooled seminal bacteria (10% saline suspension) were inoculated into equal volumes of PS and PBS (control)

All were incubated at 37°C and aliquots removed at 1, 2, 4, 6, 12, 18, 24, 30 and 40 hours to be tested by CIE for activity against the 5 panel sera Bacterial cultures in Wood agar were also taken at each stage of incubation

Each of the individual bacterial types isolated from PSP were inoculated into aliquots of an individual millipore filtered antigenically non reactive seminal plasma Activity was tested at 1, 1 1/2, 2, 3, 4, 6, 10 and 18 hours using K228 serum

Protease commercially prepared from *B. Subtilis* (Sigma) in 20 µg amounts was incubated at 37°C in 1 ml of non reactive millipore filtered individual seminal plasma Aliquots were tested for antigenic activity at 1 hourly intervals for 3 hours and hourly for 3 hours then 4-hourly for 18 hours

Both millipore filtered PSP and CFE were heated for 10 min at 46°C, 70°C, 80°C and 100°C Antigenic activity was tested using 2 PSP CFE positive panel sera by CIE

To investigate the possible loss of labile antigenic component in PSP the 3 well CIE technique was used to test unheated PSP and PSP heated to 70°C

Screening of test sera for activity to heat stable components in PSP PSP was heated to 70°C for 15 min and used as antigen to screen 116 unselected subfertility sera

Bacterial-unassociated component(s) in seminal plasma Using the 3 well CIE procedure antigenic component(s) of the PSP distinct from bacterial antigens was sought in reactions with the 5 panel sera The existence of an additional component was confirmed by incubating a pooled suspension of the 7 bacterial types in a millipore filtered seminal plasma from one healthy individual and tested for activity in 3 well CIE at 6, 12, 18 and 24 hours against each of the 7 bacterial cell free extracts The 3 well system was also used to determine the number of antibody specificities in 4 of these sera to PSP

Removal of bacterial antigens in PSP by absorption with chicken antisera to these antigens Two male chick

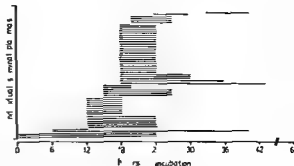


Fig 2 Incubation of spouse seminal plasmas Each line indicates the period of CIE antigenic activity of each of 60 spouse seminal plasmas under incubation at 37°C

were each immunised intravenously with 100 µl of a 10% pooled suspension of the 7 bacterial types and bled 1 and 2 weeks later The sera were reactive against each of the 7 bacterial antigens to a titre of 1/8 The sera were pooled and the globulin precipitated with sodium sulphate at a final concentration of 14%

PSP which was already heated to 70°C for 15 min was absorbed with the chicken globulin in a ratio of one part PSP to 8 parts globulin The mixture was kept at room temperature for 6 hours and then at 4°C overnight followed by centrifugation at 3000 r.p.m. for 20 min to remove the precipitate The absorbed PSP was tested by CIE to determine whether bacterial antigens had been completely removed The presence of excess chicken antisera in the PSP was also checked using CFE

The absorbed PSP was then used to demonstrate the presence of the bacterial-unassociated component(s) in 3 well CIE using the 5 panel sera and CFE

Serum reactivity to absorbed PSP All the study sera were tested by CIE for activity to absorbed PSP undiluted and 1/8 diluted

RESULTS

In the entire group of 182 subfertile patients 78 (42.9%) eventually became pregnant during the period of study 1969-1976 These comprised 49 (41.9%) in the primary group and 29 (44.6%) in the secondary group (Fig 1)

Reactivity of freshly obtained seminal plasma No antigenic activity was demonstrated when seminal plasma from a fresh ejaculate was tested against 30 of the subfertile patients' sera which were known to react with modified seminal plasma

Effects of incubation on spouse seminal plasmas Activity was observed in each of the 60 spouse seminal plasmas following varying periods of incubation at 37°C (Fig 2) Activity was detected in 4 before incubation but with the remaining and more showed reactivity at 6, 12, 18, 24, 30, 36, 42 and 48 hours

Table 1 Reactivity of sera from subfertile and comparison fertile groups to pooled seminal plasma (PSP), pooled bacterial cell free extract (CFE), heated PSP and PSP absorbed with chicken antisera to bacterial antigens (absorbed PSP) tested by counterimmunoelectrophoresis (CIE)

Study groups	No tested	Antigens used in CIE			
		PSP positive (%)	CFE positive (%)	Heated PSP positive/total (%)	Absorbed PSP positive (%)
<i>Subfertile</i>					
<i>Primary</i>					
Non pregnant	68	55 (80.9)	56 (82.4)	21/36 (58.3)	19 (77.9)
Subsequently pregnant	49	41 (83.7)	41 (83.7)	14/35 (40.0)	11 (77.4)
<i>Secondary</i>					
Non pregnant	36	33 (91.7)	32 (88.9)	17/23 (77.3)	22 (61.1)
Subsequently pregnant	29	27 (93.1)	25 (86.2)	8/23 (34.8)	6 (70.7)
Total subfertile	182	156 (85.7)	154 (84.6)	60/116 (51.7)	58 (31.7)
Comparison fertile	127	80 (63.0)	68 (53.5)	Not tested	15 (11.8)

hours with a peak frequency at 18 hours. The last 2 to become reactive did so at 24 and 35 hours. The duration of activity varied from 8 to 40 hours with 6 hours being the commonest period (40 specimens—66.7%).

Serum reactivity to PSP The titre of activity in PSP was 1/512 when tested against K228 serum. The reactivity of sera from the study groups are shown in Table 1. One hundred and fifty six (85.7%) in the combined subfertile group and 80 (63.0%) in the comparison group were positive. The difference was statistically significant ($\chi^2=14$, $p<0.0005$). The frequency of positives in the fertile subgroups is also shown in Table 1. There was however no difference between the cases that subsequently achieved pregnancy and those that remained non pregnant. The antibody activity of the sera was low and did not exceed 1/8.

Demonstration of immunoglobulin activity in CIE reactions Precipitin bands were seen when immunoglobulin preparations of 3 CIE positive subfertile sera were tested on CIE with PSP.

Bacteriological studies on seminal plasma Numerous bacteria were seen on microscopy of PSP. Seven bacterial types were isolated from it. They were identified as *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus subtilis*, non haemolytic and haemolytic diphtheroids, mucoid *E. coli* and *Streptococcus faecalis*.

Serum reactivity to bacterial cell free extract Using K228 serum the titre of activity of the CFE was 1/32. The frequency of positives is shown in Table 1 with 154 (84.6%) of the subfertile group

being reactive compared with 68 (53.5%) of the fertile women. Again the difference between the 2 groups was significant ($\chi^2=35.7$, $p<0.0005$). There were no differences between the subfertile subgroups (Table 1). The antibody activity of the sera was again low, never exceeding a titre of 1/4.

Immunochemical relationship of antigens in PSP and CFE Using the 3 well CIE procedure multiple bands were seen between the antibody well and each of the 2 antigen wells containing PSP or CFE. Their number varied from 2 to 9 and formed complex patterns which were difficult to interpret. Some of the bands were continuous across the antigen wells. There were other bands formed with PSP which were non identical with CFE and which we refer to as bacterial unassociated component(s).

Characterisation of antigens in seminal plasma No antigenic activity was detected in the sterile PSP even after 40 hours of incubation. Sterility was confirmed by the absence of bacterial growth on culture.

In the CFE experiment in which CFE was incubated with PS or PBS no rise in the titre of activity was observed. Bacterial growth was also absent.

In the PS seeded with pooled seminal bacteria activity was detected from the first hour and persisted for 40 hours. No antigenic activity was seen in the PBS control.

Inoculation of aliquots of a freshly prepared seminal plasma separately with each of 7 bacterial types resulted in appearance of antigenic activity varying from 1/2 hour to 6 hours of incubation regardless of the type of bacterium used.

Antigenic activity was not produced by incubation

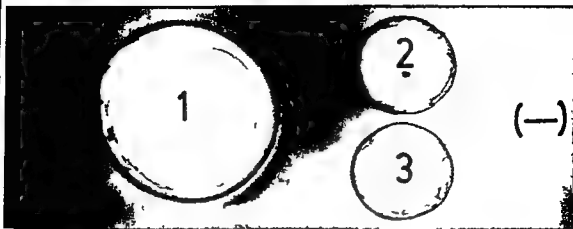


Fig. 3 Immunochemical relationship of precipitin systems by 3-well CIE. Test serum (1) pooled seminal plasma (PSP) (2) and pooled bacterial cell free extracts (CFE)

(3) A line of partial identity is seen between PSP and CFE. A line of non-identity (arrowed) is shown between PSP and CFE.

ing a bacterial enzyme protease (extracted from *B. subtilis*) with seminal plasma.

When PSP was heated to 56°C its titre of activity was unchanged at 1/256 before and after heating. However the titre fell to 1/64 after heating to 70°C. It remained the same even at 100°C. Similarly heating CFE to 56°C gave no change in its activity (titre 1/16). But heating to 70°C and above resulted in partial loss of activity (titre 1/4).

The loss of the heat labile component in PSP was also shown using the 3 well CIE procedure. A precipitin line which was observed with unheated PSP disappeared when PSP was heated to 70°C. However other precipitin lines remained indicating thermostable antigen antibody systems.

Activity of test sera to heat stable components in PSP. Using PSP which was heated to 70°C as antigen 116 sera from the subfertility group were screened. 60 (51.7%) were positive (Table I).

Evidence for bacterial unassociated component(s) in seminal plasma. The component(s) additional to bacterial antigens which was seen in earlier 3 well CIE experiments involving 159 sera from the subfertile group was again observed using 4 of the panel sera. A typical result is shown in Fig. 3. Three of these sera showed 2 antibody specificities; the fourth had only one. An example to demonstrate these specificities is given in Fig. 4.

Following incubation of sterile seminal plasma with the pool of 7 bacterial types the antigens produced were studied using 3 well CIE. Two bands were seen but the more cathodally situated was

non identical with each of the 7 bacterial cell free extracts. These bands were obtained at all stages of incubation. The cathodally placed band was identified as the bacterial unassociated component(s).

The same component(s) was also observed when absorbed PSP was tested on 3 well CIE. There was non identity with CFE.

Serum reactivity to absorbed PSP. The results of serum activity to absorbed PSP are given in Table I. Only 58 (31.2%) of the subfertile group reacted as compared with 15 (11.8%) of the fertile women. The differences were again highly significant ($\chi^2 = 16.7$, $p < 0.0005$). Also the total number of patients in both primary and secondary groups who were positive and subsequently became pregnant was 17 (9.3%) as compared with 41 (22.5%) who remained non pregnant. However the differences within the primary group for the non pregnant and subsequently pregnant patients were not significant. But in the secondary group the differences within the group were statistically significant ($\chi^2 = 9.1$, $0.001 > p > 0.005$).

DISCUSSION

Previously we have reported the presence of antibodies to seminal plasma components in the sera of both infertile and fertile women using CIE (2, 3, 4). From the present study it is apparent that freshly ejaculated seminal plasma does not give precipitin bands on CIE. The

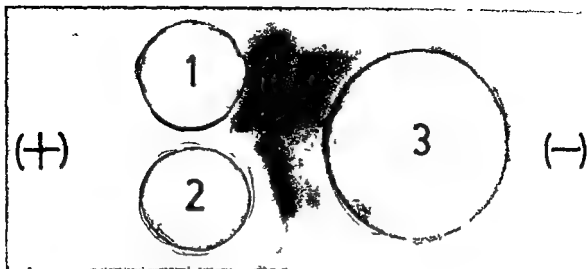


Fig. 4 Number of antibody specificities by 3 well CIE between 2 sera (1-2) and pooled seminal plasma (3). A

line of identity is seen between (1) and (3) indicating a common specificity. A second specificity is seen with (1)

genicity appears to follow incubation with bacteria. The effect can be regularly produced in any sample of seminal plasma although variation in the time of appearance and disappearance of antigenicity was observed. For the purpose of obtaining antigenic preparations of seminal plasma aliquots can be sampled at different times of incubation. This has enabled greater reproducibility of positive reactions in CIE to be achieved.

The role of bacterial in the appearance of seminal plasma antigenicity is not understood. Seven bacterial types were isolated from PSP. While the sources of these bacteria were not precisely known they were presumably located around the external genitalia. Incubation of seminal plasma with each of the seven types of bacteria resulted in the appearance of bacterial antigenicity and of antigenicity unassociated with bacteria and therefore presumably associated with seminal plasma. Intact bacteria were required for the production of plasma associated antigenicity. During incubation antigens of bacterial origin were released into the seminal plasma and contributed to the total antigenicity of the plasma. Some of these antigenic components disappeared on heating to 70°C a feature which was exploited in the preparation of plasma antigenic material unassociated with bacterial antigenicity. Further removal of bacterial antigenicity was achieved by absorption with chicken antisera raised against the seven bacterial types.

Attention has previously been given to a possible role for bacteria in subfertility. Riley & Masters (12) have cultured various bacterial types from the male partners of infertile couples. Teague et al. (14) found a decrease in motility and viability of spermatozoa in the presence of *E. coli*. Quesada et al. (11) reported an association of genital infection and spermagglutinating antibodies in infertile men. The studies reported here lend support to the significance of bacteria in the development of seminal plasma antigenicity and hence in the induction of immunity to plasma antigens.

The occurrence of immunity to modified seminal plasma antigens was investigated in 187 subfertile patients and in 127 fertile women. The subfertile group had no detectable organic cause for their subfertility. Using PSP as the source of antigen 146 (85.7%) of the sera from subfertile women were positive on CIE compared with 80 (63.0%) of the fertile group. Evidence that these high frequencies were in part attributable to anti-bacterial immunity was obtained by the lower frequencies of positive reactions using heat-treated antiserum absorbed PSP. Positive reactions were detected in 58 (31.2%) of the subfertile group compared with 15 (11.8%) among the fertile women.

When the positive reactions were analysed in relation to subgroups of subfertile patients it was apparent that the higher frequency of positivity in the total group was largely due to positive reactions in 22 (61.1%) of 36 secondary non-pregnant patients

The other 3 subgroups showed a lower frequency of positivity (22.7%–27.9%) which was approximately two-fold greater than that in the fertile women.

The occurrence of pregnancy in primary and secondary subgroups was similar [49 (41.9%) and 9 (44.6%) respectively] suggesting that at least in the patients in this study a history of pregnancy was not of prognostic significance in terms of the probability of subsequent pregnancy. Within the primary and secondary subgroups the frequency of CIE positive reactions using absorbed PSP in women who subsequently became pregnant was also similar being 11 (22.4%) of 49 and 6 (20.7%) of 9 respectively.

Has a positive CIE reaction any clinical relevance? Among women in the primary subfertile group there was no relation between CIE reactivity and subsequent occurrence of pregnancy. By contrast in patients in the secondary subfertile group the frequency of subsequent pregnancy was lower in the CIE positive patients. Thus it does appear that CIE positivity may be of clinical significance in the evaluation of previously pregnant subfertile women. Since the nature of the presumed antigen-antibody system detected by CIE is unknown it is premature to speculate whether the immunity to modified seminal plasma antigen as revealed by a positive CIE reaction is causally related to reduced fertility.

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INCIDENCE OF STERILITY IN WOMEN OPERATED ON IN CHILDHOOD FOR PERFORATED APPENDICITIS

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Abstract Of 80 women who had appendicectomy in childhood 78 were reviewed with reference to sterility. Thirty nine of them had been operated on for acute perforated appendicitis (APA) and 39 for acute non perforated appendicitis (ANPA). There was no statistically significant difference in the incidence of sterility between the two groups. In the APA group the incidence of sterility differs from that in sterility models with the same age composition. It may therefore be advisable for young girls to have appendicectomy at the least indication.

One of the causes of mechanical sterility is pelvic inflammation. Pelvic peritonitis thus leads to sterility in 78-94% of cases (2). Whether acute perforated appendicitis is followed by an increased incidence of mechanical sterility remains obscure (4-6). This problem has not previously been investigated by following up cases of childhood appendicitis. The present study was therefore undertaken to evaluate the incidence of infertility in females who had been operated on in childhood for acute perforated appendicitis.

MATERIAL AND METHOD

The material consisted of 80 females admitted to the surgical departments A and B, Københavns Amtssygehus in Glostrup during the period 1959-1970. In October 1975 a questionnaire was sent to all of them with questions about pregnancies, unsuccessful attempts for more than one year to conceive, medical examinations because of infertility and intraperitoneal operations during the period of observation. A total of 78 (98%) replied and were included in the study. A distinction was made between infertile and fertile patients and those with unknown fertility. Regarded as infertile were patients who had been involuntarily sterile for more than one year. Fertile were those who had been pregnant after operation. The patients with unknown fertility had neither wanted to nor tried to conceive. The series was distributed into two groups: one contain-

ing patients operated on for acute perforated appendicitis (APA) the other containing patients operated on for acute non perforated appendicitis (ANPA). The former group included 39 females ≤ 15 years at the time of surgery and ≥ 0 years at the time of follow-up. The latter group consisted of 39 females selected as follows: following each patient operated on for APA the first consecutive patient of the same age (± 1 year) operated on for ANPA entered the group. In this way we obtained two groups of the same composition with regard to age and time of observation (Table I).

None of the patients had undergone intraperitoneal surgery prior to the appendicectomy which in all cases was performed by the same method (no peritoneal lavage or drainage and closing of the wound without the use of local antibiotics). In all cases the diagnosis was verified macroscopically as well as microscopically. Only patients with APA were given systemic treatment with antibiotics in the form of either oxytetracycline or penicillin in combination with streptomycin.

RESULTS

It appears from Table II that there were 5 sterile patients in the APA and 3 in the ANPA group. The incidence of sterility was 26 and 14% respectively. There was no statistically significant difference in the incidence of sterility between the groups.

Table I Mean age at the time of operation and at follow up

Standard deviation stated in parenthesis

	Acute perforated appendicitis (APA)	Acute non-perforated appendicitis (ANPA)
Age at operation (years)	11.6 (2.6)	11.3 (2.9)
Age at follow-up (years)	23.5 (2.7)	23.5 (3.0)

Table II Incidence of sterility in the groups acute perforated appendicitis (APA) and acute non perforated appendicitis (ANPA) calculated from the sum of sterile and fertile patients

	Number of patients			Incidence of sterility (95% confidence limits)
	Unknown fertility	Fertile	Sterile	
APA	20	14	5	26 (9-51)
ANPA	17	19	3	14 (3-35)

($\chi^2 0.4$ $P < 0.7$) The 8 infertile patients had tried to conceive for mean 25 months (S.D. 8.7) and at follow up their mean age was 23.6 years (S.D. 2.7). Two patients in either group had been subjected to investigations because of infertility. In the APA group aerobic bacterial culture from the peritoneal cavity was carried out in 31 patients with a positive result in 28 (90%).

In the APA group one or more postoperative complications occurred in 13 patients (33%). Ileus developed in 1, intraperitoneal abscess in 10 and wound infection in 5. In the ANPA group postoperative wound infection developed in 1 patient. In each group 2 patients had undergone intraperitoneal surgery during the period of observation; none of these patients were sterile.

DISCUSSION

The effects of peritonitis can impede the migration of the ovum from the ovary to the tube and act as a potential cause of sterility. The most common cause of this peritoneal factor in infertility is pelvic sepsis (2).

In addition to the peritonitis that develops when the appendix perforates postoperative complications are likely to increase the risk of peritubal adhesions. In our series the incidence of postoperative complications does not differ from that found in other studies (1, 5).

Powley (4) reviewed 33 patients in whom appendicectomy was complicated by pelvic peritonitis or the formation of a pelvic abscess. They had all been under 40 years at the time of surgery. The incidence of sterility was found to be high.

Thompson & Lynn (6) reviewed 37 patients all of whom had been under 20 years of age when they

underwent surgery for perforated appendicitis. These authors did not find the incidence of sterility to be significantly increased but concluded that infertility is a potential sequel to perforated appendicitis.

In contrast to the above mentioned two studies our material consisted of children who only were ≤ 15 years at the time of operation and who had not previously undergone intraperitoneal surgery. We could not demonstrate any statistically significant difference in the incidence of sterility between the groups APA (26%) and ANPA (14%).

In models of female sterility the incidence of infertility in the age group 22-5 years is stated to be 3.8-6.3%. In our study only the ANPA group comes within this range of infertility (cf. confidence limits Table II). This would suggest an increased tendency to infertility in women who have been operated on for perforated appendicitis although this group of patients does not differ from that operated on for uncomplicated appendicitis.

In conclusion the present investigation shows that in women who have been subjected to appendicectomy in childhood there is no significant difference in the incidence of sterility between those who have been operated on for APA and those operated on for ANPA. However, as compared with sterility models with the same age composition the incidence of sterility in the APA group was higher than expected. It may therefore be advisable for young girls to be subjected to appendicectomy whenever there is any indication to do so.

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INTRA URETHRAL AND INTRA VESICAL PRESSURE IN CONTINENT WOMEN

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Abstract Two age groups of continent women were examined regarding the pressures in the urethra and the bladder using a standardized recording technique with a microtransducer catheter elaborated in 1974 by Ulmsten & Asmussen. The two groups of women (mean age 38 and 54 years respectively) were examined concerning their urethral pressure profile at every 100 ml bladder filling until the maximal bladder capacity had been reached. The functional length of the urethra and the bladder pressure were the same for all women but the maximal urethral pressure decreased significantly with age (mean 61.5 and 39 mmHg in the two groups respectively). At increasing bladder filling only the younger group was able to respond with an increase in the maximal urethral pressure. The bladder volume at which severe incontinence occurred was significantly higher in the elderly group.

Urinary stress incontinence is a frequent symptom in the female. In search of the genesis of this disorder two parameters in particular have been penetrated, namely the functional length of the urethra and the urethral closure pressure. Changes in these are according to Lapedes (10), Hodgkinson (5) and Enhörning (4) of great relevance for the pathology and treatment of stress incontinence. To evaluate the findings in the incontinent female it is necessary to consider what characterizes the continent condition. Therefore simultaneous urethrocystometry including the measurement of the urethral pressure profile has been performed on 22 continent women. One group of women of fertile age has been compared with another group of early postmenopausal age. All of them were examined with the urethral pressure profile at rest at increasing bladder volume in order to study whether the urethral pressure varied with the degree of bladder filling. In addition an analysis has been made of the

relationship between the urethral pressure profile and the urethral anatomy.

Similar pressure studies have been made by Asmussen & Ulmsten in 1975 (1) with the same pressure recording equipment as used in this investigation. Two groups of continent women with approximately the same age distribution as in this paper were examined although with a constant bladder volume. The urethral pressure and the functional length of the urethra were reported to differ between the groups with a higher pressure and greater length in the younger group. The greater length was probably at least partly caused by a lower bladder pressure in these women.

Enhörning (4) in 1961 also reported a higher urethral pressure in younger continent women. He however used somewhat less precise equipment.

MATERIAL

Two groups of volunteers were examined: fourteen elderly women with a mean age of 54 years ($r=64-48$) and 8 much younger women with a mean age of 38 years ($r=36-19$). The mean parity in the two groups was 1.2 ($r=3-1$) and 0.25 ($r=1-0$) respectively. The criteria for participation were: complete absence of incontinence symptoms or other disturbances in the urinary tract, a normal gynecological status and a negative urinalysis. The younger women had no symptoms from the uro-genital tract. The elderly women were hospitalized for curettage because of postmenopausal bleeding. In all cases the urethrocystometry was performed before the operation. All women were informed of the aim of the investigation and gave their verbal consent.

METHOD

Simultaneous urethrocystometry was performed with a catheter in which two microtransducers had been en-

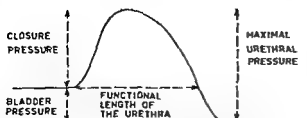


Fig 1 The curved line represents the recorded pressure from the bladder along the urethra to the external urethral meatus

closed one at the tip of the catheter and the other 60 mm proximally (Millar Instrument Inc. Houston, USA). For technical details concerning the recording equipment, see the catheter, the amplifier and the calibration unit, see the thorough description by Ulmsten, Asmussen, Lindström 1975 (13). For the urethral pressure profile measurements, the catheter was drawn through the urethra with a velocity of 2.5 mm/s by a synchronous motor. During withdrawal of the catheter, the end transducer continuously recorded the intra-vesical pressure, while the proximal transducer recorded the pressure continuously along the urethra. By electronic subtraction, the closure pressure was also registered. Thus, the urethral pressure, the bladder pressure and the urethral closure pressure were recorded simultaneously at every moment during the examination. The technique has a high precision and good reproducibility and has been published (2).

Experimental performance

The patients were examined in the lithotomy position. Residual urinary volume was measured. 100 ml of 0.9% saline solution at 32°C was instilled in the bladder. The catheter was inserted transurethral so far into the bladder that both microtransducers were located intraluminally. The catheter was attached to the arm of the withdrawal apparatus, and three consecutive urethral pressure profiles were recorded. A baby feeding catheter (outer diam. 1.32 mm) was inserted transurethral into the bladder without touching the pressure transducers, and another three profiles were recorded. Three urethral profiles were obtained at every 100 ml increase in bladder volume until 500 ml (the younger group) or until severe tenesmi occurred (the elderly group). At the maximal bladder volume, another three urethral pressure profiles were measured without the baby feeding catheter. The infusion velocity was 25 ml/min.

At a bladder volume of 200 ml, the urethral profile was also measured at holding urine. Light and severe tenesmi were noticed as well as the absence of leakage at repeated coughs at every 100 ml increase in bladder volume. Afterwards, the patients were given urinary antiseptics prophylactically (methenaminhippurat Hiprex®).

Definitions and interpretations

Definitions according to the ICS Standard Committee 1975 (7).

Urethral pressure = The pressure within the urethra recorded in relation to the atmospheric pressure.

Intra-vesical pressure = The pressure within the bladder recorded in relation to the atmospheric pressure.

Urethral pressure profile = Continuous recording of the intraluminal pressure throughout the entire length of the urethra.

Urethral closure pressure = The intra-urethral pressure minus the bladder pressure.

The functional length of the urethra = The part of the urethra where the intraluminal pressure exceeds the bladder pressure.

How the parameters are measured from the records is shown in Fig 1.

Three urethral pressure profiles were measured at every 100 ml bladder volume, but only the third one was counted at the statistical analysis, the reason being that some women had more sensitive urethras than others. Involuntary movements and contractions of the pelvic floor might distort the urethral pressure profile. Repeated recordings of 10 consecutive profiles under the same conditions showed that these were almost identical from the third one onwards.

RESULTS

The elderly group

The mean functional length of the urethra decreased from 32 (s.d. 4.4–22.5) to 28 (s.d. 3.9–19) mm when the bladder volume was increased from 100 to 500 ml ($p < 0.01$). Because the urethral pressure profiles were individual, the differences between the functional length at 100 and 500 ml bladder volume were analyzed with Student's *t* test.

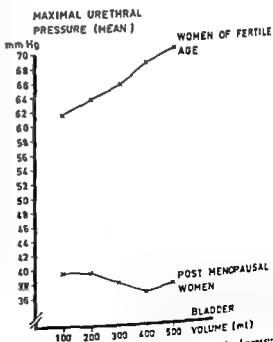


Fig 2 The change in the mean maximal urethral pressure at increasing bladder filling in the two groups.



Fig 3 Comparison between a typical urethral pressure profile recorded in a woman of fertile age (A) and a postmenopausal woman (B)

The mean maximal urethral pressure did not change with increasing bladder volume. The mean values were approximately 39 mmHg (see Fig 2). In that part of the urethra where the pressure was highest there were pressure variations synchronous to the patient's breath and pulse. The amplitudes of these pressure variations were 5–10 and 2–5 mmHg respectively. As the transducer moved along the urethra during the measurement of pressure profiles these amplitudes diminished. The intra-individual variations in urethral maximal pressure never exceeded 5 mm when measured from repeated pressure profiles.

The mean bladder pressure increased from 8.5 ($r=13-5$) to 12 ($r=16-7.5$) mmHg when the bladder volume was increased from 100 to 500 ml. A slow continuous pressure increase was seen in all patients ($p<0.01$).

Light tenesmus occurred at a mean bladder volume of 350 ml ($r=550-200$). Severe tenesmus occurred at a mean bladder volume of 600 ml ($r=800-400$). The mean total bladder capacity was 600 ml ($r=800-550$).

The urethral pressure profile at holding urine was recorded in all patients at 200 ml bladder volume. Only 5 patients could hold urine without contracting the abdominal muscles at the same time thereby changing the urethral pressure profile. Changes in both the functional length and the maximal pressure were seen when these patients' profiles at rest and at holding urine at the same bladder volume were compared.

The younger group

The mean functional length of the urethra decreased from 32.5 ($r=37-27$) to 28 ($r=31-21$) mm when bladder volume was increased from 100 to 500 ml ($p<0.01$).

The mean maximal urethral pressure increased from 61.5 ($r=82-43$) to 70.5 ($r=90-43$) mmHg ($p<0.01$) (see Fig 2).

The breath and pulse synchronous pressure variations had an amplitude of 5–10 and 5–10 mmHg respectively. Two patients did not increase their maximal urethral pressure at increasing bladder volume. These were the only ones who did not feel severe tenesmus at 500 ml bladder volume.

The mean bladder pressure increased successively in all patients from 8.5 ($r=10-7.5$) to 13 ($r=15-10$) mmHg with increasing bladder volume ($p<0.01$).

Light tenesmus occurred at a mean bladder volume of 190 ml ($r=250-150$). Severe tenesmus occurred at a mean bladder volume of 470 ml ($r=600-300$). The mean total bladder capacity was 500 ml ($r=600-400$).

No patient in this group could hold urine without simultaneously contracting the abdominal muscles.

DISCUSSION

Independent of age the urethral pressure profile in the continent female was characterized by a rapid increase in pressure proximally. This part of the urethra is to be regarded as an anatomical continuation of the urinary bladder (11). In the middle part of the urethra there was a pressure plateau approximately 15 mm long where the pulse and breath synchronous pressure variations were clearly seen (see Fig 3). The pressure in this part of the urethra is probably maintained by smooth and semicircular striated muscles (6, 8, 9, 12, 14). In the distal fibrous part of the urethra the pressure quickly dropped to the atmospheric pressure.

The decrease in the functional length of the urethra as the bladder is filled is partly a consequence of the increasing bladder pressure. Mostly there was an additional shortening of the functional length exclusively engaging the upper part of the urethra. The distance from the end of the urethral pressure plateau to the only fixed point of the profile (meatus urethrae externum) seemed to be independent of bladder volume and bladder pressure. Thus the additional decrease in length solely engaged the upper urethra. A plausible explanation of this phenomenon is that the upper urethra shrinks under the weight of the filled bladder.

The maximal urethral pressure in the elderly group did not alter as the bladder was filled. Thus neither an increase nor a decrease in pressure was seen. It has been shown (3) that the activity of the sympathetic fibers of the hypogastric nerves increases successively during bladder filling. Since the upper urethra is partly innervated by sympathetic nerves, an increase in urethral pressure would have been expected. This was the case only in the younger group. The largest increase in urethral pressure occurred between 300 and 500 ml bladder volume. Another discrepancy between the two groups was that the urethral pressure was much higher in the younger group, probably due to better vascularization. This hypothesis is supported by the higher amplitude of the pulse synchronous variations in this group (Fig. 3).

The higher bladder capacity and the later sensation of tenesmus in the elderly women were unexpected results. They might be explained by an impaired sensibility and proprioception in the bladder and the urethra, caused by diminished vascularization with increasing age and a changed hormonal influence. Further ageing probably brings about degenerative atrophic changes in the bladder, so that its capacity decreases.

Light and severe tenesmus were not correlated to any changes in the parameters in the elderly group. Severe tenesmus was not associated with any unced increase in bladder pressure.

At holding urine the bladder is displaced ventro cranially by the contraction of the muscles of the pelvic floor. This is probably achieved mainly by the pelvic fascia (bladder ligaments) being stretched when the levator muscles contract. Hereby the functional length of the urethra increases uniformly. The maximal urethral pressure also increased. Sometimes the increase involved not only the urethral pressure plateau but also the part of the urethra proximal to this plateau. Since the pressure increase affected the profile only partly and on different sites from case to case, it seems likely that the levator muscles also exert a direct effect on the urethra and that this pressure increase is not accomplished by the semicircular striated muscle fibers in the urethral wall.

CONCLUSION

The aim of this investigation has been to compare the pressure and pressure variations in the urethra

and the bladder at increasing bladder volume at rest in two groups of continent women. One group consisted of women of fertile age and the other of women of early postmenopausal age. The functional length was the same in both groups. It has further been shown that the functional length of the urethra changed with changing bladder volume. There was a significant shortening of the functional length at increasing bladder filling. This shortening involved only the upper part of the urethra and was partly caused by an elevated bladder pressure, probably also by a slight anatomical shortening. The maximal urethral pressure decreased with age. In young women the urethra had the ability to react with a pressure rise on increasing bladder volume. This ability was lost with age.

The comparison between women of fertile and postmenopausal age showed that the functional urethral length and the bladder pressure behaved similarly at bladder filling. The bladder capacity was increased and the sensation of severe tenesmus appeared later in the elderly group, probably because of an impaired vascularization.

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MANAGEMENT OF CARCINOMA IN SITU OF THE UTERINE CERVIX BY SELECTIVE LOCAL EXCISION

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Abstract Local excision of severe dysplasia or carcinoma in situ of the uterine cervix by punch biopsies was the treatment in 366 cases. In 61% cervical cytology reverted to negative or slight atypia. 153 patients have been followed from 1 to 7 years. Failures to remove the entire lesion were discovered within the first year of follow up in all cases but 10. Complications were negligible and no patients have subsequently developed invasive cancer of the cervix. Selective local excision as an alternative to other minimally traumatic techniques e.g. cryotherapy is discussed. Although the failure rate is slightly higher with local excision an obvious advantage is the availability of all removed tissue for histopathologic diagnosis. Thus the risk of overlooking microinvasive disease is reduced to a minimum.

A conservative attitude towards the treatment of severe dysplasia and carcinoma in situ (CIS) of the uterine cervix prevails in the Scandinavian countries (2, 8, 10). The dominant procedure is therapeutic conization. Recently the necessity of this procedure in all cases has been questioned. It has been suggested that in selected cases local excision under colposcopic control might be an equally effective method (1). The small and inexpensive resources needed for this office procedure which is carried out without anesthesia is of potential advantage for both the patient and her physician.

This is a report on the results obtained by local excision of severe dysplasia and carcinoma in situ in 366 selected patients.

PROCEDURES AND MATERIALS USED

The patients in this series were selected from the general screening program for the detection and prevention of cervical cancer which has been in operation in the County

of Malmöhus in Southern Sweden since 1967. Colposcopy is performed routinely in all cases of abnormal cytology. When cytologic and colposcopic findings were compatible with severe dysplasia or carcinoma in situ in a large atypical transformation zone direct therapeutic conization was carried out as a rule. The results of this procedure have been reported previously (2).

Patients with small areas of colposcopically abnormal epithelium in the transformation zone were treated by local excision. This was performed by means of one or several punch biopsies using Schubert biopsy forceps. Endocervical curettage was added when a small lesion extended into the cervical canal. In a few cases when the lesion was confined entirely to the endocervix curettage was the sole procedure. Bleeding following the excision was usually slight and was controlled by local application of Nelex® solution. To prevent late postoperative hemorrhage and to enhance the healing process by acidification Nelex® vaginal suppositories were given every other day for a 3-week period. During the same period coital abstinence was recommended. Only patients with a diagnosis of severe dysplasia or CIS confirmed by histopathologic examination were included in the study.

Postoperative cytologic smear control was started after 3 months. When only benign cells or cells showing slight atypia were found smears were repeated every 3 months for one year and subsequently every 6-12 months. In cases of evidence of persistent dysplasia or CIS therapeutic conization was usually performed. However in a few cases the extension of the abnormal epithelium was still considered so limited that a second local excision was tried. Those results are not included in this report.

RESULTS

Total numbers of women screened and of those with a histopathologic diagnosis of severe dysplasia or CIS are listed in Table I. From this table it is apparent that the patients selected for treatment with local excision are fairly evenly distributed between the different 5 year age classes. The

Table I Cytologic screening program 1967-1974
County of Malmöhus Sweden

Year of birth	Total no screened	Total no with severe dysplasia/CIS	Total no selected for local excision	%
1913-19	11 604	82	35	43
1920-24	8 971	98	39	40
1925-29	10 460	147	89	61
1930-34	10 162	180	90	50
1935-39	10 850	127	74	58
1940-	8 587	64	39	81
Total	58 634	698	366	52

tion in the older women. Obviously conservative management is less critical with increasing age. In total 52% were considered candidates for the procedure.

The results indicated by postoperative cytology and by time of follow up are presented in Table II. The total time of follow up is given for patients whose cytology reverted to negative or to slight atypia. For those with persistent or recurrent severe dysplasia or CIS the interval preceding its detection is reported. In 223 patients (61%) there were no signs of residual or recurrent severe dysplasia/CIS. Of these 153 have been followed for 1-7 years. In a few cases one or several smears read as benign/slight atypia were later followed by smears indicating more severe abnormalities. However it is apparent that in the great majority of cases failures to remove all areas of severe intra-epithelial neoplasia were detected within the first 6-12 months of follow up. Only 10 patients with postoperatively negative or slightly atypical smears followed for one year or more later developed more severe neoplastic conditions. Of these 8 were diagnosed during the second year of follow up and only 2 subsequently.

In Table III the data have been broken down with respect to the different age-classes. No distinct differences were found. In fact the highest rate of residual disease occurred in the oldest and the youngest patients.

Of 70 patients with a follow up of less than one year and postoperatively negative or slightly atypical cytology 16 (23%) were negative and 54 (77%) had slight atypia. The latter group includes inflammatory changes and mild dysplasia (Table IV). The administrative computer program used for the

screening does not permit a similar breakdown of the cases followed for one year or more. However the distinct impression is that early postoperative slight atypia often disappears gradually and therefore could represent an operative effect such as repair. No patients have developed invasive cancer during the period of observation.

DISCUSSION

Cytologic screening programs aimed at the detection and prevention of cervical cancer are widely used in many parts of the world. Since the peak incidence of CIS occurs in the age group 25-34 years (4, 5) and that of dysplasia even earlier, screening and re-screening will result in a gradual increase of the proportion of young women found to have cervical intra-epithelial neoplasia. This emphasizes the need for therapeutic approaches which inflict minimal trauma on the reproductive tract. Nevertheless cure rates must be acceptable and the risks of overlooking existing early invasive cancer or predisposing the patient for its later development must be negligible.

With a conservative attitude regardless of the technique is used, willingness of the patient to undergo regular cytologic examinations post-operatively and during several years to come is a prerequisite. She must also be able to cope with the possibility that not all disease may be removed at the first attempt. In our program follow up is administered by a rather complex computer system described previously (3). The problem of losing patients to follow up is practically non-existent.

The efficacy of cervical conization in our patient population has been reported previously (7). Our

Table II Cervical intra epithelial neoplasia treated by local excision (overall results)

Time after biopsy	Postoperative cytology	
	Negative/slight atypia	Severe dysplasia/CIS
<6 months	54	13
6-12 months	16	10
1-2 years	31	8
2-3 years	30	1
3-4 years	153	10
4-5 years	15	1
>5 years	10	1
Total	223	143

Table III Cervical intra epithelial neoplasia treated by local excision (results in relation to age)

Year of birth	Total no	Postoperative cytology			
		Negative/ slight atypia follow up <1 year	Negative/ slight atypia follow-up >1 year	Severe dysplasia/CIS Total no	Occurring after 1 year
1913-1919	35	7	8	20	3
1920-1924	39	5	21	13	2
1925-1929	100	11	51	27	2
1930-1934	90	17	45	28	2
1935-1939	74	16	24	34	1
1940-	39	14	4	21	-
Total no	366	70	153	143	10

have reported on the successful use of outpatient procedures such as electrocauterization and cryosurgery (6-7, 12). One potential disadvantage of these techniques is that only a limited portion of the neoplastic epithelium is removed for histopathologic examination. We and others have clearly demonstrated that even in experienced hands colposcopy has limitations and will fail to pick up a certain number of early invasive lesions (2).

In this study local excision with one or multiple punch biopsies and possibly endocervical curettage is described as an alternative form of therapy in selected patients with small atypical transformation zones. Even with random biopsies a certain number of patients will revert to normal cytology (9, 11). In this series with colposcopically directed biopsies 61% of the cases reverted to essentially normal cytology. These results are not compatible with those reported with electrocauterization or cryosurgery. However the technique offers two potential advantages. Firstly all tissue is made available for histopathologic examination thus reducing to a minimum the risk of overlooking microinvasive disease. Secondly the depth of excision is

generally greater than can be achieved with the other two techniques.

Failure to remove all neoplastic growth is in most instances detected within the first 6 months of follow up. It is uncertain whether the recurrences seen later than one year after local excision represent true recurrences or progression of persisting slight atypia. However with adequate follow up there is no indication that local excision as a means of controlling cervical intra epithelial neoplasia is hazardous to the patient. On the contrary she will be rendered the benefit of preserved fertility and a minimal risk of complications from the treatment.

ACKNOWLEDGEMENT

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Table IV Cervical intra epithelial neoplasia treated by selective local excision (distribution of negative and slightly atypical smears within first year after local excision)

Time after biopsy	Negative	Slight atypia
6 months	10	44
6-12 months	6	10
	16	54

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CASE REPORT

SEVERE HAEMORRHAGE FROM THE NON PREGNANT UTERUS AS A RESULT OF CICATRICIAL NECROSIS AFTER CERVICAL CAESARIAN SECTION

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Abstract Two cases are reported where severe uterine haemorrhage caused by cicatricial necrosis occurred one month after cervical caesarian section. The aetiology, diagnosis, therapy and prophylaxis are discussed and reference is made to previously reported data.

Severe haemorrhage from a necrotic scar in the uterus months or years after caesarian section is a complication rarely reported and it is our impression that the complication, as the few available reports suggest, is rather unknown.

Within a short interval of time two cases with this type of haemorrhage occurred in our department. Treatment by hysterectomy was required.

admission the patient was complaining of severe constant pain at the site of the symphysis. Blood pressure 100/80, pulse rate 96. Under general anaesthesia the uterus was cautiously evacuated on the suspicion that remnants of placental tissue might be present. To all appearances the cavity was smooth and without remnants of tissue. Still a necrotic tissue specimen measuring 1×3 cm and not resembling placental tissue was removed. The haemorrhage could not be arrested and laparotomy was performed. The entire cicatrix was found to be necrotic and subtotal hysterectomy according to Crobach was indicated. According to an estimate the overall loss of blood amounted to 3000 ml. The postoperative course was uneventful. Whether or not abnormal coagulation might be in evidence was not tested.

Histological examination of the uterus showed unspecific inflammatory processes in the myometrium reaction to foreign body and signs of earlier haemorrhage. On examination of the tissue specimen the endometrium and the myometrium were found to be partially necrotic. (Signed: Per Moeller Nielsen.)

CASE REPORTS

No 1

The patient was a 23 year-old woman, gravida 2, para 2. Caesarian section had been performed four years earlier in her first pregnancy and in the second pregnancy caesarian section was performed at term on the 18th January 1972 because the foetus was apparently large.

At the time of the second operation the lower uterine segment was of normal appearance. It was opened by transverse section and digital dilatation. A live healthy boy, weight 4200 g, was delivered. The uterus was sutured in two layers using continuous chrome catgut no. 1 while continuous plain catgut was used in close the serosa. Complications of a technical nature were not encountered during surgery. Lochia was normal. Antibiotics were not given. The patient was discharged from hospital in good health 10 days after parturition. Gynaecological examination at the time of discharge from hospital showed normal conditions.

The patient was re-admitted as an emergency on the 17th February 1977 (30 days after parturition) because of severe vaginal haemorrhage which had started two hours earlier. Minor bleeding with the lochia had persisted throughout the first two weeks after surgery but in the following two weeks there had been no vaginal bleeding. At

No 2

The patient was a 25 year-old woman, gravida 1, para 1 who was admitted at term on the suspicion of a contracted pelvis indicating induction of labour. As the labour even though stimulated failed to progress satisfactorily laparotomy combined with cervical caesarian section was performed on the 28th August 1973. The lower uterine segment was found to be of normal appearance. It was opened by transverse incision and digital dilatation. A live healthy boy, weight 4300 g, was delivered. The incision into the uterus was torn during the procedure, the tear extending into the parametrium to the right, resulting in the formation of a minor haematoma. The uterus was sutured in two layers using continuous chrome catgut no. 1. Plain catgut no. 0 was used for the suture of the tear. The temperature was elevated at a maximum up to 38.4°C throughout the initial three postoperative days. Antibiotics were not given. Lochia was normal. The patient was discharged from hospital in good health 10 days after surgery. Gynaecological examination at the time of discharge from hospital showed normal conditions.

The patient was re-admitted as an emergency on the

21st September 1973 (33 days after parturition) because of the sudden onset of severe vaginal haemorrhage. Minor bleeding had been an everyday occurrence after her discharge from hospital. She had no pain. Blood pressure 100/70 pulse rate 100. On the suspicion that remnants of placental tissue might be present in the uterus the latter was cautiously evacuated while the patient was under general anaesthesia and a few specimens of necrotic tissue were removed. The cavity was found to be of smooth appearance. The haemorrhage could not be arrested however and laparotomy was performed. The tissue surrounding the suture in the uterus was found to be necrotic and bleeding from several vessels was noted. Total hysterectomy was performed. The overall loss of blood amounted to 6000 ml. The postoperative course was uneventful. Whether or not abnormal coagulation might be in evidence was not tested.

Histological examination of tissue obtained at evacuation of the uterus showed a post partum endometrium and decidual remnants but the presence of remnants of placental tissue could not be reliably demonstrated. Examination of the uterus did not reveal any true pathological post partum changes (Signed Per Moeller Nielsen).

DISCUSSION

Hansford & Weed (1) reported in 1953 for the first time that cervical caesarian section might be followed by severe vaginal haemorrhage about one month after surgery the phenomenon had been observed in two patients who were admitted by way of emergency on account of such vaginal haemorrhage caused by a necrotic cicatrix in the uterus. Heys (2) described two cases of a similar nature which he had seen personally and he cited two unpublished reports on the phenomenon. Reports on a further two cases of cicatricial necrosis and haemorrhage appeared in 1963 and 1974 (3, 6). Stewart & Evans (7) have recently described one case where the haemorrhage apparently originated in a venous space at the corner of a thin but otherwise intact cicatrix and Manson (4) described a case where the haemorrhage originated in an aneurysmal dilatation of a venous sinus on the posterior wall of the uterus though the cicatrix remained intact.

There is every reason to believe that the haemorrhage described in the present paper was caused by necrosis of the cicatrix and subsequent erosion of the vessels. The necrotic tissue specimen obtained from patient no. 1 was undoubtedly a fragment of the cicatrix. In some of the above cited cases the necrosis may have turned into a genuine dehiscence. Cicatricial necrosis may certainly not result always in severe haemorrhage occasionally it may provoke an asymptomatic dehiscence or a dehiscence

which at least remains asymptomatic until a later pregnancy. Pedowitz & Schwartz (5) declared on the basis of findings in a prospective study that the incidence of asymptomatic dehiscence ranged around 8% after cervical caesarian section.

It appears from the case reports that the loss of blood may be serious and accordingly it is essential to have the diagnosis established and treatment instituted without delay. Treanor (8) found that the incidence of puerperal haemorrhage of a severity requiring hospitalization was 0.51% after parturition via the vaginal route while it was only 0.27% after delivery by caesarian section.

With a view to determining whether or not a haemorrhage should be presumed to originate in a cicatrix Hansford & Weed and Heys set up the following criteria:

- 1) that intra uterine digital palpation and cautious evacuation do not suggest other possible causes or

- 2) that a defect at the site of the cicatrix can be demonstrated by palpation.

In the cases reported by Hansford & Weed the patients had been successfully treated with intra-uterine tamponade exclusively while laparotomy had been performed in all of the other cases reported. Hysterectomy is definitely the most reliable measure but other possibilities must be taken into consideration. In the case reported by Keane (3) the cicatricial defect was resutured and the anterior branch of the internal iliac artery was ligated. Bilateral ligation of the anterior branches or of the main internal iliac artery may also serve to bring the haemorrhage under control. Even simple excision of the cicatrix and resuture may be sufficient. As regards the two cases discussed in the present paper however hysterectomy was considered the only possible measure.

The question to arise is whether the prevention of a cicatricial necrosis may depend on the technique used for surgery?

It applies to both operations that continuous chrome catgut suture no. 1 of the two layers of the uterine wall was used. Suture of the internal layer being through the entire uterine wall while suture of the external layer was rather superficial. Pedowitz & Schwartz studied the various techniques used for suture but they could not give priority to one type over the others. Some authors are of the opinion that continuous suture may exert a particularly traumatic effect. On the other hand as a haemo-

static means it is the most valuable type of suture the perfect haemostasis thus produced is especially emphasized by Pedowitz & Schwartz. The latter authors recommend the use of an inverting technique for suture thereby preventing penetration of endometrial tissue into the myometrium.

Continuous catgut suture in two layers of the uterus has been used in all of the above cited cases. We believe that it is the most generally used technique and reports on a few cases with the combination of haemorrhage and use of continuous suture do not justify the conclusion that such a technique is unsuitable. The incision into the uterus must be made in the correct site (1). Cervical tissue is known to be incorporated into the lower uterine segment during labour and incisions at a cervical site may increase the risk of necrosis. Vascularization of the cervical tissue is poor as compared with that of the lower uterine segment per se. It has been pointed out by Ross & Galliford (6) that risks of incorrect incision are parallel with the magnitude of dilatation during the preceding labour if in progress. Pedowitz & Schwartz observed that the risk of a development of cicatricial necrosis is not intensified even if patients after operation run an elevated temperature. Furthermore they observed that routine administration of antibiotics after surgery was of no apparent importance for the healing of the wound.

In the patients discussed in the present paper the severe haemorrhages occurred at almost identical stages after surgery namely on days 30 and 33 respectively. The cicatricial necrosis observed by the above mentioned authors had been demonstrable after intervals covering from 21 to 53 days after parturition which is similar to our findings.

With a view to inspecting the integrity of the scar Heys recommended the performance of hysterosalpingography of patients whenever major bleeding occurs after caesarian section.

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BOOKS RECEIVED

Clinics in Obstetrics and Gynecology: The Menopause vol 4 no 1 (edited by Robert H Greenblatt and John Studd) 67 pp Price per single copy £7 50 subscription price £15 00 (3 issues) W B Saunders Company Ltd England 1977

Can be recommended as a good survey with references up to 1976

IS

Guide to the Collection and Transport of Virological Specimens by C R Madeley Geneva World Health Organization 1977 40 pages Price Sw fr 10 — US\$4 70 Geneva 1977

Recommended

IS

Breast Cancer Trends in research and treatment (European Organization for Research and Treatment of Cancer EORTC Monograph Series vol 2 edited by J C Heuson W H Matthei and M Rozenweig) 343 pp 1976

Based on the first Breast Cancer Working Conference of the European Organization for Research on Treatment of Cancer 1975 Virology immunology experimental models endocrine aspects palliative treatment curative treatment epidemiology and screening and organization of breast cancer research are dealt with Recommended to all gynecologists interested in breast cancer

IS

An Obstetric Tragedy (Editor Franco Crauz) 77 pp Price £3 50 William Heinemann London 1977

A collection of documents concerning Princess Charlotta Augusta's delivery in 1817 and her death five hours after the delivery of a stillborn son

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A good collection of current methods which can be recommended to all departments of obstetrics and gynecology

IS

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Proceedings from the Sero Symposium in Freiburg 1975 A good collection of basic as well as clinical research concerning ovulation

IS

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This classical book has been brought up to date and can still be recommended

IS

ANNOUNCEMENT

111rd Reinier de Graaf Symposium on non luteal ovarian function

The third Reinier de Graaf Symposium will be held September 4-6 1978 in Maastricht one of the oldest towns but site of the youngest Medical Faculty in the Netherlands

It will be dedicated to

a Ovarian follicular morphology and function from intra uterine life until the onset of puberty

b Non luteal ovarian morphology and function during the period of reproductive capacity

c Ovarian morphology and function after reproductive age

The Reinier de Graaf lecture will be delivered by Dr Hannah Peters Copenhagen

For further information please contact Prof E.J. Lind van Hall Department of Obstetrics and Gynaecology Leiden State University Leiden The Netherlands

Letter to the editor

Dear Sir

In the article Chorioangioma with hydramnios and intrauterine fetal death

by M. Kuoth et al in

Acta Obstet Gynecol Scand 55 279 1976 there is

an aspect that I found very interesting. Dr Nothmann & associates mention that the placenta was large oedematous measuring $21 \times 20 \times 4$ cm weighing 150 g

Placental hypertrophy has been reported as associated with chorioangioma both of the diffuse variety by Potter (2) and of the localised by Earn & Penner (1) and by Sen (3)

The case reported by Dr Kuoth and his associates would appear to be unique in having both localised and diffuse chorioangiomas in association with placental hypertrophy

I would in particular like to ask Dr Kuoth if they found immature oedematous villi with small peripheral non sinusoidal capillaries as was in the last case (3). Certainly there does not seem to be any

indication of depressed placental function in these cases even though villous oedema mimicking hydatidiform mole is sometimes seen. Nevertheless the perinatal mortality is high probably as indicated due to a circulatory overload on the fetal cardiovascular system. The mechanism causing placental hypertrophy and proliferation of immature villi remains obscure.

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